Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Clippinger *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA] have also described a decision tree and potential key events that can be used to design pathway-based approaches for *in vitro* testing of inhalation exposures.

Table [SEQ Table * ARABIC]. Potential Methods for Evaluating Chemicals in the Surfactant Category.

Level of Biological Organization	*Key Events*	*TestingTier*	*In Vitro Assay*	*Test System*
Molecular Initiating Events (MIEs)	Interaction with pulmonary surfactant	*II*	*In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant interaction, *e.g.*, as described by Sorli *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA]
	Interaction with cell membrane and	II	Hemoglobin Denaturation Assa	• Hemoglobin denaturation assay, *e.g.*, as described by Hayashi *et al.* (1994) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1994</Year><RecNum>14838</RecNum><DisplayText>[95]</DisplayText><record><rec-number>14838</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596732926">14838</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hayashi, T.</author><author>Itagaki, H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Kato,

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	cell membrane components and interaction		y, Liposome Assay, and *In Vitro /Ex Vivo* Irritation Assays	S.</author></authors></contributors><auth-address>Shiseido Production Research Center, 1050 Nippa-cho, Kohoku-ku, Yokohama 223, Japan.</auth-address><titles><title>Multivariate factorial analysis of data obtained in seven in vitro test systems for predicting eye irritancy</title><secondary-title>Toxicol In Vitro</secondary-title><alt-title>Toxicology in vitro : an international journal published in association with BIBRA</alt-title></titles><periodical><full-title>Toxicology in vitro : an international journal published in association with BIBRA</full-title><abbr-1>Toxicol In Vitro</abbr-1></periodical><alt-periodical><full-title>Toxicology in vitro : an international journal published in association with BIBRA</full-title><abbr-1>Toxicol In Vitro</abbr-1></alt-periodical><pages>215-20</pages><volume>8</volume><number>2</number><edition>1994/04/01</edition><dates><year>1994</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0887-2333 (Print)
0887-2333</isbn><accession-num>20692908</accession-num><urls></urls><electronic-resource-num>10.1016/0887-2333(94)90185-6</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] • Liposome assay, *e.g.*, as described by Kapoor *et al*. (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><RecNum>14834</RecNum><DisplayText>[96]</DisplayText><record><rec-number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300">14834</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors></contributors><auth-address>Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalmol Vis Sci</secondary-title><alt-title>Investigative ophthalmology & visual science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></periodical><alt-periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</number><edition>2009/01/27</edition><keywords><keyword>Conjunctival Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques, Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><keyword>Humans</keyword><keyword>*Liposomes</keyword><keyword>Luminescent Measurements</keyword><keyword>Models,

				Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-Active Agents/*toxicity</keyword></keywords><dates><year>2009</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electronic-resource-num>10.1167/iovs.08-2980</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] • *In vitro/ex vivo* eye irritation tests for penetrance, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD TG 492) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[97]</DisplayText><record><rec-number>14803</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>43, https://www.oecd-ilibrary.org/docserver/9789264242548-en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><volume>492</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>], Bovine Corneal Opacity and Permeability Test (OECD TG 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14802</RecNum><DisplayText>[98]</DisplayText><record><rec-number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719">14802</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeability Test Method For Identifying i) Chemicals Inducing Serious Eye Damage And ii) Chemicals Not Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846-en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><volume>437</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], Isolated Chicken Eye Test (OECD TG 438) [ADDIN EN.CITE

				<EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14804</RecNum><DisplayText>[99]</DisplayText><record><rec-number>14804</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for identifying I) chemicals inducing serious eye damage and II) chemicals not requiring classification for eye irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203860-en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><volume>438</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], *etc.*
Cellular Level Events (CLEs)	Loss of membrane integrity/general cytotoxicity	*II*	*In Vitro /Ex Vivo* Cytotoxicity Assays	• *In vitro/ex vivo* eye irritation tests for cytotoxicity, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD TG 492) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[97]</DisplayText><record><rec-number>14803</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithelium (RhCE) test method for identifying chemicals not requiring classification and labelling for eye irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>43, https://www.oecd-ilibrary.org/docserver/9789264242548-en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><volume>492</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>], Bovine Corneal Opacity and Permeability Test (OECD TG 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14802</RecNum><DisplayText>[98]</DisplayText><record><rec-number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719">14802</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeability Test Method For Identifying i) Chemicals Inducing Serious Eye Damage And ii) Chemicals Not Requiring Classification For Eye Irritation Or Serious Eye

				Damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846-en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><volume>437</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], Isolated Chicken Eye Test (OECD TG 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14804</RecNum><DisplayText>[99]</DisplayText><record><rec-number>14804</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for identifying I) chemicals inducing serious eye damage and II) chemicals not requiring classification for eye irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203860-en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><volume>438</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], *etc.*
		II		• Cell membrane integrity test (LDH-cytotoxicity assay), cell viability assays (*e.g.*, MTT, resazurin [ADDIN EN.CITE ADDIN EN.CITE.DATA], and ATP), TEER [ADDIN EN.CITE ADDIN EN.CITE.DATA], or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCVAM, 2006) [ADDIN EN.CITE <EndNote><Cite><Author>ICCVAM</Author><Year>2006</Year><RecNum>14805</RecNum><DisplayText>[101]</DisplayText><record><rec-number>14805</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044231">14805</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ICCVAM</author></authors></contributors><titles><title>In vitro Cytotoxicity Test Methods for Estimating Starting Doses for Acute Oral Systemic Toxicity Testing</title><secondary-title>ICCVAM Test Method Evaluation Report</secondary-title></titles><periodical><full-title>ICCVAM Test Method Evaluation Report</full-title></periodical><pages>334, https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf</pages><volume>NIH Publication No. 07-4519</volume><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]

Tissue or Organ Level Events (OLEs)	Tissue level events	III	Human organotypic Airway Cultures	• 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (*e.g.*, EpiAirway™, MucilAir™, SmallAir™, EpiAlveolar™, *etc.*) using the cell membrane integrity and viability assays described under cellular level events [ADDIN EN.CITE ADDIN EN.CITE.DATA]
	Tissue level events	III	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test, *e.g.*, as described by Hess *et al*. (2016) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and Neuhaus *et al*. (2017, 2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA]

MIEs

There may be multiple ~~AOP~~MOAs that would be relevant to the Surfactant Category. The MIE for a proposed MOA ~~AOP~~ under development is the interaction of a substance with lung surfactant, which may lower the surface tension and disrupt lung surfactant function [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Sorli *et al*. (2017) [ADDIN EN.CITE ADDIN EN.CITE.DATA] developed an *in vitro* lung surfactant interaction assay that specifically measures whether a substance alters the surface tension of pulmonary surfactant. The assay was initially developed for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle that may be relevant for some types of surfactants.

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The proposed MIE for another AOP relevant to surfactants is direct interaction with AEC or pulmonary cell membranes, which may be followed by cytotoxicity. While the hemoglobin denaturation and liposome assays and *in vitro* eye irritation assays do not directly measure effects on membranes of AEC, these assays have been shown to be useful screening approaches for determining the ability of surfactants to interact with cellular membrane components and cell membrane penetration. For example, Hayashi *et al.* (1995) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1995</Year><RecNum>14833</RecNum><DisplayText>[105]</DisplayText><record><rec-number>14833</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539110">14833</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hayashi, T.</author><author>Itagaki, H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Sato, Y.</author><author>Suzuki, Y.</author></authors></contributors><auth-address>Shiseido Research Center, Yokohama, Japan.</auth-address><titles><title>Hemoglobin denaturation caused by surfactants</title><secondary-title>Biol Pharm Bull</secondary-title><alt-title>Biological & pharmaceutical bulletin</alt-title></titles><alt-periodical><full-title>Biological & Pharmaceutical Bulletin</full-title><abbr-1>Biol. Pharm. Bull.</abbr-1></alt-periodical><pages>540-3</pages><volume>18</volume><number>4</number><edition>1995/04/01</edition><keywords><keyword>Chromatography, High Pressure Liquid</keyword><keyword>Circular Dichroism</keyword><keyword>Hemoglobins/*chemistry</keyword><keyword>Irritants/pharmacology</keyword><keyword>Protein Denaturation/drug effects</keyword><keyword>Sodium Dodecyl

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Sulfate/pharmacology</keyword><keyword>Spectrophotometry</keyword><keyword>Structur
e-Activity Relationship</keyword><keyword>Surface-Active
Agents/*pharmacology</keyword><keyword>Taurine/analogs &
derivatives/pharmacology</keyword></keywords><dates><year>1995</year><pub-
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num>10.1248/bpb.18.540</electronic-resource-num><remote-database-
provider>NLM</remote-database-
provider><language>eng</language></record></Cite></EndNote>] showed that charged surfactant molecules can interfere with charged side chains of the hemoglobin protein. These interactions led to disruption of the three-dimensional (3D) structure of hemoglobin, causing a change in light absorbance that can be measured. Increasing concentrations of SDS and sodium lauroylmethyltaurate (LMT; CASRN 4337-75-1) were tested in this assay and showed concentration dependent increases in hemoglobin denaturation, which correlated with irritation effects in the Draize eye test [ADDIN EN.CITE ADDIN EN.CITE.DATA].

The liposome assay can be used to assess disruption of the lipid bilayer of the membrane from interaction with surfactant chemistries. Kapoor *et al*. (2009) [ADDIN EN.CITE
<EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><RecNum>14834</RecNum
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address>Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalmol Vis Sci</secondary-title><alt-title>Investigative ophthalmology & visual science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></periodical><alt-periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</number><edition>2009/01/27</edition><keywords><keyword>Conjunctival Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques, Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><keyword>Humans</keyword><keyword>*Liposomes</keyword><keyword>Luminescent Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-Active Agents/*toxicity</keyword></keywords><dates><year>2009</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electronic-resource-num>10.1167/iovs.08-2980</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] measured the release of calcein dye from liposomes following exposure to various surfactants and showed a positive correlation with these findings and data from the Draize eye test. The hemoglobin denaturation

and liposomal assays were both optimized and validated against eye irritation data; therefore, these assays may provide an opportunity to evaluate the effects of surfactants on the respiratory tract. Further *in vitro* testing of known surfactants with existing data alongside new chemical substances will help benchmark the results. Nonetheless, these assays are useful for understanding the potential toxicity of a new surfactant substance to AEC or pulmonary cell membranes.

The use of *ex vivo* eye irritation studies may provide indirect measures of surfactants on cell membranes, which may be relevant to the effects observed from comparator substances in the respiratory tract. For example, Bader *et al*. (2013) [ADDIN EN.CITE <EndNote><Cite><Author>Bader</Author><Year>2014</Year><RecNum>14807</RecNum><DisplayText>[107]</DisplayText><record><rec-number>14807</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044694">14807</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bader, J.E.</author><author>Norman, K.G.</author><author>Raabe, H.</author></authors></contributors><titles><title>Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability Assay</title><secondary-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</secondary-title></titles><periodical><full-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</full-title></periodical><pages>https://iivs.org/wp-content/uploads/2018/08/iivs_poster_predicting-ocular-irritation-of-surfactants-using-the-bovine-corneal-opacity-and-permeability-assay.pdf</pages><dates><year>2014</year></dates><urls></urls></record></Cite></EndNot

e>] reported that the Bovine Corneal Opacity and Permeability (BCOP) assay was effective at demonstrating that nonionic (*i.e.*, octylphenoxypolyethoxyethanol), anionic (*i.e.*, SDS), and cationic (*i.e.*, BAC) substances cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. The permeability score was more predictive of eye irritation than the ocular opacity score for octylphenoxypolyethoxyethanol and SDS, whereas with BAC, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation of opacity and permeability measures of surfactants tested in the BCOP may be helpful with elucidating toxicity to AEC or pulmonary cell membranes.

In addition, information on the potential of a substance to cause skin irritation (*e.g.*, OECD TG 439 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14808</RecNum><DisplayText>[108]</DisplayText><record><rec-number>14808</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044884">14808</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed Human Epidermis Test Method, In vitro Skin Irritation</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>26, https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1596045726&id=id&accname=guest&checksum=2580E92A5C8

89D0DD65599260E7866D3</pages><volume>439</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]) and/or skin corrosion (*e.g.*, OECD TG 431 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14809</RecNum><DisplayText>[109]</DisplayText><record><rec-number>14809</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044976">14809</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>29, https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1596045820&id=id&accname=guest&checksum=E3EE55CBAAFAF0432EAD109F1B39ECF0</pages><volume>431</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]) *in vitro*, can provide supporting evidence of the potential for a substance to cause similar irritant or corrosive effects in respiratory tract cells. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, demonstrated by DDAC that causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 [ADDIN EN.CITE <EndNote><Cite><Author>Sigma-Aldrich</Author><Year>2020</Year><RecNum>14810</RecNum><DisplayText>[110]</DisplayText><record><rec-number>14810</rec-number><foreign-keys><key app="EN" db-

id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045132">14810</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sigma-Aldrich</author></authors></contributors><titles><title>Safety Data Sheet, Product name: Didecyldimethylammonium chloride, Version 8.1, Revision Date: 03/28/2020, Print Date: 05/29/2020</title></titles><pages>9, https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

Cellular Level Effects

In vitro/ex vivo assays can be used to assess ~~key events on the~~ cellular level effects ~~in AOPs relevant~~of chemicals in ~~to~~ the Surfactant Category (see Supplemental Table 1 in Clippinger *et al.*, 2018 [ADDIN EN.CITE ADDIN EN.CITE.DATA]). For general cytotoxicity ([REF _Ref46931271 \h * MERGEFORMAT]), cell lines are available that are known to be sensitive to the effects of surfactants. Use of the BALB/c 3T3 NRU cytotoxicity test to reduce animal testing by estimating starting doses for acute oral toxicity testing has been reviewed and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and is an OECD guidance document [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The surfactants with known inhalation toxicity (*e.g.,* octylphenoxypolyethoxyethanol, oleoyl sarcosine, DDAC, or BAC) should be tested in parallel with the new chemical substance to benchmark the results, thereby providing reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

Tissue or Organ Level Effects

Based on the results of testing cellular level key events, it may be necessary to perform additional testing. Human and animal airway epithelia are composed of multiple cell types that each have specialized functions, making the use of 3D co-culture assays more physiologically relevant than 2D monoculture systems. Thus, several human organotypic airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems. Two commonly employed systems are EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively.

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Organotypic airway cultures, such as EpiAirway™ and MucilAir™, [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[112]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33,

https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain homeostasis for months in culture. Because of these characteristics, these human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Dosimetry models such as the RDDR or MPPD can be used to predict the anatomical area and internal amounts delivered in various regions of the respiratory system for humans under the target inhalation exposure scenario for the given use case. Different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. MucilAir™ provides a 3D co-culture model of cells from nasal, tracheal or bronchial sites, and SmallAir™ provides a co-culture model of cells from small airways. EpiAirway™ is composed of a co-culture of normal human tracheal/bronchial epithelial cells, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts (available with and without macrophages).

Exposure of respiratory tract 3D co-culture models to aerosols at the air liquid interface (ALI) using an *in vitro* exposure system, such as those available from Vitrocell® Systems, provides an exposure more comparable to real-life scenarios for inhaled aerosols. The tradeoff has been a lower throughput compared to *in vitro* two-dimensional exposure systems; however, 3D tissue models and ALI exposure systems are now available in a 96-well format. Dilution in medium

and interaction with medium components does not occur in the ALI exposure systems as in submerged culture systems. The respiratory tract 3D co-culture models are more physiologically relevant because there is an interaction of the aerosol with a mucus or surfactant layer, as in humans.

Exposures of these organotypic cultures at the ALI can be combined with other assays for assessing cell function and viability in an AOP approach. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays (such as MTT, resazurin, or ATP assays), have all been reported for use with these cultures. Further, multiple assays can be performed on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant octylphenoxypolyethoxyethanol at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, provided dosimetry models are available for translation of the internal dose achieved under culture conditions to an equivalent inhalation exposure for the human scenario of interest. Examples of *in vitro* dosimetry models to predict particle doses for submerged cell culture include the *In vitro* Sedimentation, Diffusion and Dosimetry model (ISDD) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and the *In vitro* Sedimentation, Diffusion and Dissolution Dosimetry (ISD3) model [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Significant progress has been made toward achieving the objectives to use high-throughput *in vitro* assays and computational models to evaluate potential adverse effects of chemical exposures [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[16, 115]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite><Cite><Author>NRC</Author><Year>2017</Year><RecNum>14812</RecNum><record><rec-number>14812</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045703">14812</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Using 21st Century Science to Improve Risk-Related Evaluations, Washington, D.C., The National Academies Press</title></titles><pages>200, https://doi.org/10.17226/24635</pages><volume>ISBNs: Ebook: 978-0-309-45351-6; Paperback: 978-0-309-45348-

6</volume><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. To translate the effects to higher levels of biological organization, a battery of assays with varying complexity and physiological relevance may be needed. The 3D human organotypic airway cultures add evidence to an AOP approach and increase confidence in the physiological relevance to humans.

Precision-cut lung slices (PCLS) provide an additional method to develop key event data using *ex vivo* cultures of human or rodent lung slices. The PCLS can be used to measure multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often used in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema [ADDIN EN.CITE <EndNote><Cite><Author>Sanderson</Author><Year>2011</Year><RecNum>14814</RecNum><DisplayText>[117]</DisplayText><record><rec-number>14814</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046031">14814</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sanderson, M. J.</author></authors></contributors><auth-address>Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA 01655, USA. Michael.Sanderson@umassmed.edu</auth-address><titles><title>Exploring lung

physiology in health and disease with lung slices</title><secondary-title>Pulm Pharmacol Ther</secondary-title><alt-title>Pulmonary pharmacology & therapeutics</alt-title></titles><periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></periodical><alt-periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></alt-periodical><pages>452-65</pages><volume>24</volume><number>5</number><edition>2011/05/24</edition><keywords><keyword>Animals</keyword><keyword>Cell Physiological Phenomena</keyword><keyword>Disease Models, Animal</keyword><keyword>Humans</keyword><keyword>Lung/pathology/*physiology</keyword><keyword>Lung Diseases/*pathology</keyword><keyword>Microscopy/methods</keyword><keyword>Muscle Contraction/physiology</keyword><keyword>Organ Culture Techniques</keyword></keywords><dates><year>2011</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1094-5539 (Print)
1094-5539</isbn><accession-num>21600999</accession-num><urls></urls><custom2>PMC3168687</custom2><custom6>NIHMS296121</custom6><electronic-resource-num>10.1016/j.pupt.2011.05.001</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be evaluated. One further advantage of PCLS is that the assay can be performed on multiple species to determine inter-species variability in susceptibility.

Human PCLS, derived from, for example, rejected but otherwise healthy transplant tissue, can be used to measure cell/tissue viability, local respiratory inflammation, and physiological function. These endpoints can be measured in single and repeated exposures in a metabolically competent system within the normal architecture of the lung in a more relevant model system, replacing the need for animal testing [ADDIN EN.CITE ADDIN EN.CITE.DATA].

When human PCLS are not available, rat PCLS provide an alternate option. The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed correlation with *in vivo* LC_{50} values [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The use of rat PCLS reduce the number of animals used to conduct dose response studies, as compared to *in vivo* inhalation tests. From a rat lung (1 g), approximately 200 slices can be prepared. In general, for each test substance concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested using tissue from a single rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures *via* liquid media or, with additional adaptations, air. As such, rodent PCLS meet the goal of reducing animal testing, although dosimetry models for their translation to HEC are not yet developed. Mechanistic rodent and human PCLS studies may be conducted in parallel to understand species specific difference in toxicological effects. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations of an ~~AOP~~ MOA Approach to the Surfactant Category

A number of *in vitro* assays have been discussed as to their potential utility for assessing key events in an AOP(s) relevant to characterize the Surfactant Category. Uncertainties and limitations associated with these assays are discussed for each of the above testing systems, as well as others [ADDIN EN.CITE ADDIN EN.CITE.DATA]. It is important to consider that these assays were not systematically tested using surfactants. Nonetheless, these assays can be conducted using ~~an AOP~~ MOA approach to provide information on whether a new chemical meets the Surfactant Category criteria and/or to understand whether the new chemical may be more or less bioactive or toxic than the sub-category comparator chemicals. EPA will generally use the framework and analogue toxicity data identified in this investigation to assess potential risks from surfactants.

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In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment continues to evolve. A fit-for-purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, and domain of applicability for evaluating a new method to inform specific decisions has emerged from the regulatory science community to address the challenges posed for validation of NAMs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Such fit-for-purpose validation approaches are intended to be flexible and adaptable and to provide data sets, prediction analysis results, inference models, *etc*. in a transparent manner that enable other scientists to confirm the performance of the assays and inference models, as well as evaluate the rationale for using these assays in a specific decision context.

Once such fit-for-purpose scientific evaluations are documented, there are several ways that these assays can be used to reduce and replace animal testing. First, testing can be performed based on an AOP approach to evaluate the potency of new surfactants versus a comparator substance within the relevant subcategory that has repeated exposure inhalation toxicity data. Second, depositional data using models such as the RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

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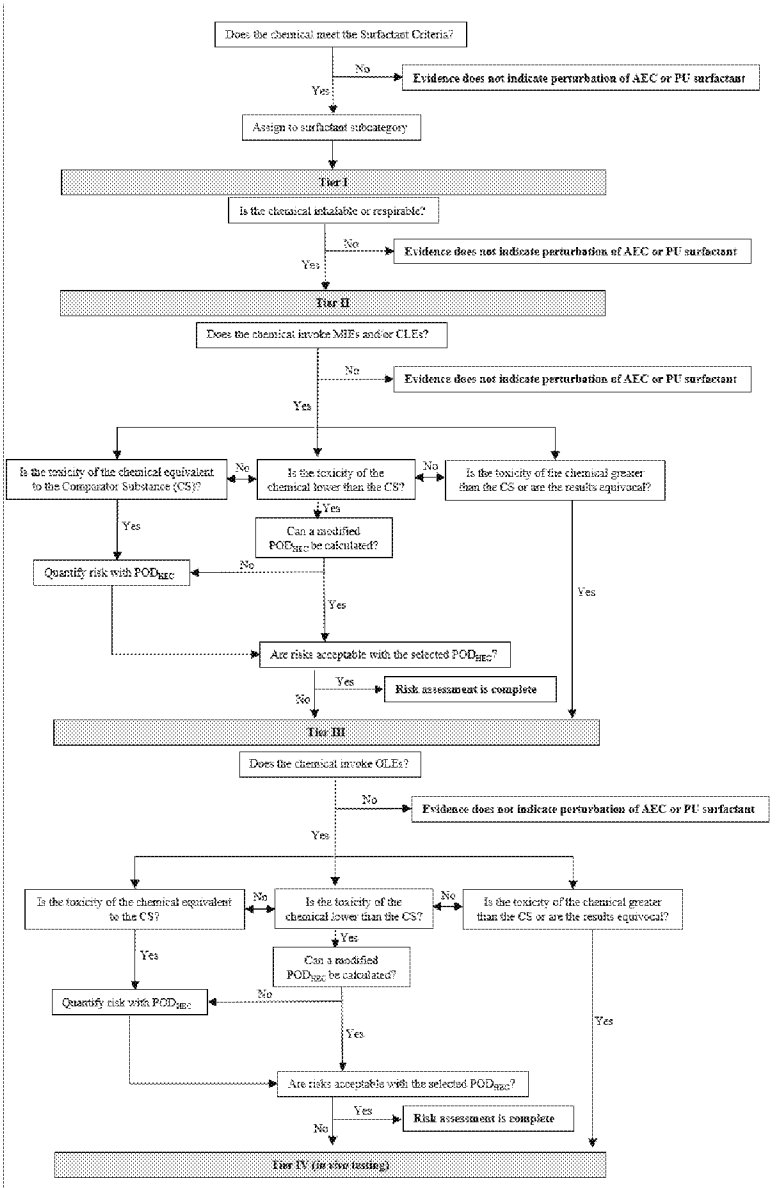
Tiered-testing Strategy

The first step in the tiered-testing strategy is to determine if the evaluated substance meets the Surfactant Criteria. If so, then assign the substance to the appropriate surfactant subcategory (nonionic, anionic, or cationic) and determine whether any of the representative subcategory chemicals may serve as an acceptable toxicological analogue for risk assessment or as a comparator substance for tiered testing. If a representative subcategory chemical is determined to be an acceptable toxicological analogue to the new chemical substance, then quantify risks using the toxicological analogue. If the MOE is equal to or greater than the benchmark MOE, then tiered testing is not required on the new chemical substance. If the MOE is lower than the benchmark MOE or if a determination cannot be made on whether any of the representative subcategory chemicals are acceptable toxicological analogues, then proceed with tiered testing using the most appropriate subcategory chemical as a comparator substance to the new chemical substance. As detailed below, the tiered-testing strategy commences with the least complex, most efficient testing methods, and at each subsequent tier, the complexity of the test system

increases, commensurate with key events in proposed AOPs relevant to the Surfactant Category, to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system. It is envisioned that both the new chemical substance and the comparator substance will be evaluated side-by-side in the NAM assays. The results of these studies may lead to the conclusion that the comparator substance is an acceptable toxicological analogue to the new chemical substance. Alternatively, the results may support that higher tiered testing is warranted, particularly when the new chemical substance has higher toxicity than the comparator substance. If *in vivo* testing is conducted, it may not be necessary to run the comparator substance in the *in vivo* tests, given that suitable inhalation studies are available on the comparator substances. A summary of the proposed tiered-testing strategy is provided in [REF _Ref48210489 \h * MERGEFORMAT] and discussed further below.



Scheme [SEQ Scheme * ARABIC]. Proposed tiered-testing strategy for general surfactants.

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Add a "Legend' to the Scheme

[PAGE]

Tier I—Physicochemical properties

Surfactants are proposed to cause a specific sequence of biological events in the respiratory tract if they are inhaled. Manufacture, processing, or use of a surfactant in an inhalable form, (*i.e.*, ≤ 100 μm aerodynamic diameter) is therefore, an initial consideration of the potential for a surfactant to cause toxicity to the respiratory tract. Particle size is an established parameter for determining inhalability/respirability of particles/droplets. Several validated test methods exist for determining potential inhalability/respirability, *i.e.*, particle size, of a new chemical substance (*e.g.*, OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><d

ates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], ISO 21501-1:2009 [ADDIN EN.CITE <EndNote><Cite><Author>ISO</Author><Year>2009</Year><RecNum>14820</RecNum><DisplayText>[121]</DisplayText><record><rec-number>14820</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046993">14820</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ISO</author></authors></contributors><titles><title>Determination of particle size distribution — Single particle light interaction methods — Part 1: Light scattering aerosol spectrometer</title></titles><pages>https://www.iso.org/standard/42728.html</pages><volume>ISO 21501-1:2009</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], OECD TG 110 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>1981</Year><RecNum>14821</RecNum><DisplayText>[122]</DisplayText><record><rec-number>14821</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047114">14821</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Particle Size Distribution/Fibre Length and Diameter Distributions; Method A: Particle Size Distribution (effective hydrodynamic radius); Method B: Fibre Length and Diameter Distributions</title><secondary-title>OECD Guidelines for the Testing of

Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>13, https://www.oecd-ilibrary.org/docserver/9789264069688-en.pdf?expires=1596047951&id=id&accname=guest&checksum=A9C13F0DFDCF2A5DD4DD39DAC64C47BC</pages><volume>110</volume><dates><year>1981</year></dates><urls></urls></record></Cite></EndNote>], and OPPTS 830.7520 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>14822</RecNum><DisplayText>[123]</DisplayText><record><rec-number>14822</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047315">14822</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Particle Size, Fiber Length, and Diameter Distribution</title><secondary-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</secondary-title></titles><periodical><full-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPPT-2009-0151-0030&contentType=pdf</pages><volume>EPA 712-C-96-037</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]). The studies shown in Table 3 suggest that the total respiratory tract may be affected from surfactants; therefore, inhalable forms ($\leq$ 100 μm) were identified as the most relevant for quantitative inhalation risk assessment. As a practical matter, a particle size cutoff of greater than 1% inhalable particles/droplets by weight (wt%), determined in a well conducted study using a

valid measurement method will generally be considered as triggering a quantitative assessment of inhalation toxicity on a new chemical substance meeting the Surfactant Criteria. EPA will generally assess the potential inhalation toxicity for a new surfactant chemical substance when the manufacture, processing or use results in greater than 1% (by weight) of the surfactant particles/droplets having a particle size of less than 100 μm. This wt% cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2017</Year><RecNum>14823</RecNum><DisplayText>[124]</DisplayText><record><rec-number>14823</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047488">14823</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>3641-3655</pages><volume>82</volume><number>8</number><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>].

If inhalable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information to determine whether a new chemical substance invokes MIEs and cellular level key events. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA is highly encouraged. In general, the testing approach in this tier should include a combination of assays, such as one that measures epithelial lining fluid/cell perturbation or pulmonary surfactant interaction/loss of function, one that measures cell membrane interaction/disruption/penetration), and one that measures loss of barrier integrity or general cytotoxicity (see [REF _Ref46931271 \h * MERGEFORMAT]). *In vitro*/*ex vivo* eye irritation studies may also be used to demonstrate cell interaction or penetration and general cytotoxicity, and *in vitro* skin irritation/corrosion studies can provide supporting evidence of possible irritant or corrosive effects in the respiratory tract.

For each assay, the comparator substance for the respective subcategory of surfactants should be tested under identical conditions. Further, the particle size distribution data may be used with dosimetry models such as RDDR or MPPD to aid with identifying the regions in the respiratory tract where deposition is expected to occur and the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Notwithstanding the uncertainties with the above assays, each may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE) for the purpose of evaluating the relative potency of the new chemical substance versus the comparator substance. Several investigations have provided insight on approaches for

accomplishing this, although with different assay systems [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In doing so, a weight of scientific evidence evaluation should be performed considering the structural features, physicochemical properties, and assay results on the new chemical substance versus the comparator substance. Based on this evaluation, the most biologically relevant endpoint(s) should be used to calculate a POD. BMD modeling may be applied to derive a $BMCL_{1SD}$ metric, as a possible metric, although the metric of one standard deviation should be used with caution [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2019</Year><RecNum>14825</RecNum><DisplayText>[126]</DisplayText><record><rec-number>14825</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048386">14825</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Transmittal of Meeting Minutes and Final Report for the Federal Insecticide Fungicide and Rodenticide Act, Science Advisory Panel (FIFRA SAP) Meeting held on December 4 and 6, 2018</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>51,https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2018-0517-0030&contentType=pdf</pages><volume>EPA-HQ-OPP-2018-0517</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] . Alternative metrics should be considered, as our understanding evolves for various *in vitro*

assays and endpoints. For example, the pharmaceutical industry has used fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc.*) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Regardless of the metric used, a justification for its selection should be provided. In those situations where data are not amenable to BMD modeling, the *in vitro* concentration tested should be determined based on the expected HEC for the appropriate subcategory (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC.

Given that the understanding of IVIVE is evolving, assay results should be interpreted in a manner consistent with the weight of scientific evidence, as noted above, while recognizing that uncertainties are often dealt with by erring on the side of conservativism. Therefore, the following initial default criteria are proposed for utilizing the assay results, and when possible, the IVIVE estimates. These criteria are consistent with EPA's approach for evaluating non-animal skin sensitization data [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14832</RecNum><DisplayText>[128]</DisplayText><record><rec-number>14832</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596244984">14832</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing (draft for public comment: April 4, 2018)</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and

Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2016-0093-0090&contentType=pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], while recognizing that the weight of scientific evidence may support an alternative interpretation to the default criteria.

The Tier II assays evaluate biologically relevant endpoints representing key events in AOPs relevant to the Surfactant Category. The results of the comparator substance and the new chemical substance in these assays provide a basis for evaluating the suitability of using the comparator substance to evaluate toxicity of the new chemical substance. Consideration should also be given to differences in the specific physicochemical properties influencing inhaled deposition (*i.e.*, MMAD, GSD, and density) between the comparator substance the new chemical. Dosimetry models such as RDDR and MPPD can be used to inform these comparisons.

If comparable toxicity is observed between the comparator substance and the new chemical substance in the Tier II assays, the POD_{HEC} from the comparator substance may be appropriately used as a toxicological analogue for quantifying the MOE. If calculated risk is acceptable stop at Tier II, otherwise proceed to Tier III.

If lower toxicity is observed for the new chemical substance versus the comparator substance in the Tier II assays, then these data should be used to determine if a modified POD_{HEC} can be quantified for the new chemical substance. If this is possible, the modified POD_{HEC} for the new chemical substance should be used for quantifying the MOE. If calculated risk is acceptable, then stop at Tier II. However, if it is not possible to calculate a modified POD_{HEC}, then the comparator substance POD_{HEC} could be used as a worse-case toxicological analogue for risk assessment. If no acceptable risk can be calculated, proceed to Tier III.

If greater toxicity is observed with the new chemical substance versus the comparator substance in the Tier II assays, suggesting risks would be identified as unacceptable, proceed to Tier III. Alternatively, there may be scientifically justified reasons for an alternative interpretation, which should be clearly articulated with the weight of scientific evidence evaluation. Otherwise, it may be necessary to proceed to Tier III.

If the results from the Tier II assays are equivocal (*i.e.*, they do not demonstrate comparable or lower toxicity of the new chemical substance versus the comparator substance), and there is no clear rationale or explanation, then proceed to Tier III testing because the data are too uncertain to make a reasoned evaluation on the potential health risks, following potential inhalation exposures.

Tier III – 3D Human Airway Models/PCLS Assay

Several testing options are available for evaluating tissue and organ level key events in an AOP relevant to the Surfactant Category. The test system employed should focus on evaluating effects

in the respiratory tract at the predicted sites of deposition (*e.g.*, ET, TB and/or PU regions), based on the particle size distribution data generated under Tier I and using RDDR or MPPD modeling. A justification for using a system(s) should be provided and may be discussed with EPA as part of a pre-notice consultation. Representative test systems include those listed in [REF _Ref46931271 \h * MERGEFORMAT].

Based on the results of the 3D-construct and/or PCLS testing, IVIVE may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[112]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM) </title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-

title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33, https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use or if the Tier III test data are not amenable for developing a POD_{HEC}, then proceed to Tier IV.

Tier IV – *In vivo* studies

Strategic *in vivo* testing may be considered as a last resort to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the comparator substances in a subcategory has representative toxicological properties to the new chemical substance, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving modified POD_{HEC}s. A pre-notice consultation meeting with EPA is strongly encouraged prior to initiating any vertebrate animal testing. This point is especially important because TSCA section 4(h)(3) indicates that any person developing information for submission under TSCA section 5 on a voluntary basis shall first attempt to develop the

information by means of an alternative test method or strategy identified by EPA before conducting new vertebrate animal testing [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>].

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with epithelial lining fluid/pulmonary surfactant and/or disrupting cellular membranes and epithelial cytotoxicity. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests should be considered:

- Step 1: OECD TGs 433, 436, and 403 address acute inhalation toxicity testing. OECD TG 433 is based on evident clinical signs of toxicity rather than death as an endpoint

(refinement) and TG 436 uses fewer of animals (reduction), and therefore, they should be considered before TG 403. Any protocol modifications should be discussed with EPA during a pre-notice consultation meeting.**

- Step 2: 5-Day inhalation study with a 14-day observation period** to address progression/resolution of effects. The OECD TG 412 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14828</RecNum><DisplayText>[129]</DisplayText><record><rec-number>14828</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048957">14828</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>28-day (subacute) inhalation toxicity study</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>23, https://doi.org/10.1787/9789264070783-en</pages><volume>412</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be used, but the exposure duration should be 5 days.

**Modifications may include pulmonary function testing (if measurable), analysis of BALF, LDH release, complete histopathological analysis of the respiratory tract and blood oxygen (pO_2) content. OECD TG 412 and OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum>

<DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>2001</Year><RecNum>14826</RecNum><DisplayText>[130]</DisplayText><record><rec-number>14826</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048712">14826</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Alarie, Y.</author><author>Nielsen, G.D.</author><author>Schaper, M.M.</author></authors><secondary-

authors><author>Spengler, B.</author><author>Samet, J. M.</author><author>McCarthy, J.F.</author></secondary-authors></contributors><titles><title>Animal Bioassays for Evaluation of Indoor Air Quality</title><secondary-title>Indoor Air Quality Handbook</secondary-title></titles><pages>23.21-23.49.</pages><dates><year>2001</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill</publisher><urls></urls></record></Cite></EndNote>].

The results of the *in vivo* testing should be used for reassessing and recharacterizing the risks of the new chemical substance.

CONCLUSIONS

The overall objective of this investigation was to develop a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. This investigation developed physical-chemical properties, *i.e.*, the Surfactant Criteria, assessors and product stewards can use for determining whether a new chemical substance can be considered a surfactant. Further, properties and characteristics are provided to divide the Surfactant Category into sub-categories for nonionic, anionic, and cationic surfactants, which is important from a toxicological perspective. A systematic literature search and review were conducted to identify data to define a Surfactant Category and substances from which PODs were identified from inhalation toxicity studies. To facilitate chemical comparisons, animal toxicity studies that could be used to derive PODs for risk assessments were identified for at least one chemical substance for each sub-category and converted to HECs using established methods developed by EPA.

Finally, a tiered-testing strategy for generating *de novo* data for new surfactant substances is provided that integrates a variety of currently available NAMs using an AOP framework. The use of this tiered-testing strategy will inform the available data on surfactants and provide greater confidence in the use of non-vertebrate testing approaches for assessing the potential risks of new chemical substances. It also offers advantages to regulators, the regulated community, and consumers because: 1) integrating NAMs into a category testing approach supports EPA, TSCA and product stewardship goals of reducing and replacing vertebrate animal testing; 2) decision analysis for higher tiered testing takes into consideration mechanistic responses, dosimetry, and exposure information; and 3) it encourages development of mechanistic data to advance the understanding of the potential inhalation toxicity of surfactants, which will drive the development of newer and safer chemistries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information file contains the following:

Section 1. Systematic Literature Review

Section 2. RDDR Modeling Outputs

AUTHOR INFORMATION

Corresponding Author

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Funding Sources

EPA sponsored the initial literature review through a government contract to SRC (68HERH19F0197 (TO#07)). The American Chemistry Council's TSCA Section 5 Testing Consortium sponsored an updated literature review by an independent third party.

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

Disclosures: TS, AMJ, KS, WI, and TRH are employed by the federal government. MPH, WK, AMK, SM, LJ, JLR, AT, and RT are employed by companies that manufacture, process, and/or use surfactants. RAB and SOS are employed by a company that represents companies that manufacture, process, and/or use surfactants. PDM and SDS work for a company that received contract funding from companies that manufacture, process, and/or use surfactants. MO and JM

work for a company that receives contract funding from the federal government. AJC and MS are employed by a company whose mission is to advance animal-free testing approaches that protect human health and the environment.

REFERENCES

[ADDIN EN.REFLIST]

The reviews for your paper are enclosed with this letter. Please consider the reviewers' comments. They have raised points that require significant consideration and revision of the manuscript before it is suitable for publication.

In addition, the authors should clarify the message of their work and decrease its length: many materials should be included as a supplementary material.

As the manuscript is considered for the Special Issue: Computational Toxicology (January 2021), we needed to shorten the usual revision time. The manuscript is due 1-Dec-2020.
Please let us know if that would be convenient for you.

Please also note that all manuscripts that deserve publication and are not submitted in time for the special issue, will be published in CRT regular issue.

When submitting your revised manuscript through ACS Paragon Plus, you will be able to respond to the comments made by the reviewer(s) in the text box provided or by attaching a file containing your response letter. In your cover letter please include your detailed responses to all of the points raised by the reviewers.

When submitting a revised manuscript, authors should include a version of the manuscript that has the Tracked Changes feature turned on, so editors and reviewers can see the revisions that were made to the original manuscript. Please upload this version of the manuscript under the tag "Supporting Information for Review Only." Authors should still indicate page and line numbers when referring to edited text in their response letter to the reviewers. An unmarked, final version of the manuscript should be uploaded under the tag "Manuscript File."

Reviewer(s)' Comments to Author:
Comment Addressed – barring any objections/edits
Comment Needs Addressing – who can address?
To Do/Check in FINAL Review

Commented [TH1]: All review/edit and return to all, esp Tala by COB Th, 11/19/20

Reviewer: 1
Comments:
The work by Henry et al. presents an analysis of the impact of surfactants upon inhalation. The topic may be interesting, however authors present the topic in such a way that is very difficult to understand the message. The work deals mainly with literature data. However, the connection between them and their selection are not clear.
Response: The authors note that the comments regarding understandability are in sharp contrast to those of reviewer #2, who was complementary regarding purpose, clarity and flow. As noted by Reviewer #2, the manuscript describes "the development of a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. The paper describes in detail the literature found on surfactants and inhalation, suggest how AOPs and NAMs can be used for risk assessment, and a scheme for how this would done."

The authors have shortened the abstract and the manuscript considerably and believe this should make clearer to the reader that this is, by design, a literature-based investigation for the purpose of establishing a TSCA New Chemicals Category, for use in evaluating new surfactant chemicals under the U.S. Toxic Substances Control Act.

Some points to address are:

1. Authors should correct spelling and typos within the entire manuscript.
   Response: The authors conducted a spell check of the Draft Proof (pdf file) and of the submitted MSWord file version of the manuscript (from which the pdf was generated) and did not identify any spelling errors in either document. We cannot check on specific instances since they are not identified by the reviewer. Nonetheless, the authors have conducted a spelling and grammar check (using MSWord) on the revised manuscript and have corrected the two typographical errors specifically identified by reviewer 2.

2. Abstract is too long, and difficult to follow. Authors should rewrite concisely the abstract. They shoul present the main aims and interests of their work, avoiding to present a detailed summary.
   Response: The authors have shortened the abstract. The revised abstract is 293 words, under the journal limit of 300 words.

3. What is the meaning of EPA? Authors should define all the abbreviation.
   Response: As defined on line 28 of the Draft Proof, EPA is the abbreviation for the U.S. Environmental Protection Agency.

4. Some information about the differences between the chosen approaches, and the information contained with REACH would be required
   Response: As indicated in the draft manuscript, the approach described is focused on assessments conducted under the U.S.'s Toxic Substances Control Act. Therefore, the authors respectfully disagree that a comparison to REACH approaches is required. However, the authors have included text acknowledging REACH and to clarify that while the approaches in the manuscript are broadly

Commented [HT2]: FROM RICK BECKER:
We'll probably have to write a note to the editor pointing out that Reviewer 1's perspectives are REACH-centric, and as clearly indicated, this paper is focused on TSCA. And that while we have addressed Reviewer 1's comments on other aspects of the manuscript, we have not broadened it to address REACH, since that is not the focus or scope of our paper. And that although the underlying scientific approaches in this article are broadly applicable, a wholly separate effort would be needed to address the specific legislative and regulatory structures of REACH.

2. I suggest we add some text acknowledging REACH, but pointing out that TSCA legislation / regulations are quite different from REACH, making it even clearer (although I think it is already clear) that this approach has been developed for TSCA, and add text along the lines of "although the underlying scientific approaches in this article are broadly applicable, a wholly separate effort would be needed to address the specific legislative and regulatory structures of REACH."

Commented [SM3]: Further REACH does only covers actual non polymeric substances, concluding this approach here is more broad

applicable for assessment of surfactants, they may or may not be applicable within the specific regulatory framework of REACH.

Furthermore, both reviewers have indicated the manuscript should be shortened. To add additional information regarding REACH would be counter to this request by the reviewers.
[Discussed and decided to also write to Editor only regarding conflicting comments regarding the manuscript being too long vs adding additional information and that REACH is really out of scope of this manuscript. ...see Rick Becker notes in the Comment Bubble]

Commented [SM4]: I think actual the manuscript has a good flow (like also the second reviewer mentioned) and we should be careful if we exclude something. I agree streamlining may be possible in some sections, but we are working with so much details that we must be careful not to lose the reader.... Reading the reviewer comments I had sometime the impression that there is a misunderstanding, e.g. see comments for particular substances and non soluble polymers

5. "A surfactant is a substance that reduces the surface tension of a liquid in which is dissolved" This definition is misleading and simple. In some cases surfactant are insoluble, eg. lipids
Response: The authors agree the definition is simple, as a starting point, it is followed by a more functional definition, i.e., regarding surface tension. Furthermore, the statement is not about water solubility and surfactants must have solubility in the solution to which they are added in order to be surface active. Nonetheless, the authors have provided an alternative definition and a reference for it in the revised manuscript, as follows:

   Any compound that reduces surface tension when dissolved in water or water solutions, or which reduces interfacial tension between two liquids, or between a liquid and a solid. *Howley's Condensed Chemical Dictionary, R. Lewis, Van Nostrand Reinhold Co., 1993, pg. 1108.*

Commented [SM5]: But it is a clear starting definition. We can integrate a better one if there is some, but I do not find the current misleading or not understandable.

Commented [SM6]: This is also ok for me

6. About toxicity of surfactants is should be included in the reference list Colloids and Surfaces B: Biointerfaces 123 (2014) 701-709 [Guzman et al., 2014; on CTAB 'a cationic surfactant', i.e., quaternary ammonium cmpd]
Response: The study referenced, Guzman et al., 2014, is a study of the "The effect of a cationic surfactant, hexadecyltrimethylammonium bromide (CTAB), on the interfacial properties of seawater" and concludes, "The results of this study underline the important role of the sea organic content in enhancing the surface-activity of surfactants, which is relevant for a deeper understand of the direct and indirect effects of these types of pollutants on the physico-chemical environment in the sea coastal areas and develop mitigation strategies." This study does not measure human or mammalian surfactant toxicity; hence, the authors do not see the linkage of this reference in the discussion of toxic effects of surfactants in conducting human health risk assessments under TSCA.

7. About inhalation toxicity, it should be included the work Current Opinion in Colloid and Interface Science 39 (2019) 24-39 [Guzman & Santini; Review article focusing on effects of particulates on biological/lung surfactants; has some good words about use of model systems to evaluate effects on lung surfactants]
[redacted] [Rick]

8. Authors should provide a definition of toxicokinetic and toxicodynamic -
Response: Toxicokinetics is generally considered the processes of absorption, distribution, metabolism, and excretion of a toxicant. Toxicodynamics is generally considered the mode of toxic action of a toxicant. The authors have provided these general definitions parenthetically at first occurrence of these terms in the revised manuscript (i.e., page XX INTRO)

9. About the change of mechanical properties of the lung surfactant layer, the reference The Journal of Physical Chemistry C 119 (2015) 26937-26947
Response: The reference, Guzman et al., 2015, looks at the effects of carbon nanoparticles on the primary lung surfactant, Dipalmitoylphosphatidylcholine. Carbon nanoparticles do not meet the

traditional definition of a surfactant and are outside the boundary conditions in this paper which covers commercial surfactants. The authors note that there is a companion paper in this special issue of Chem Res Tox that looks at the effects of poorly soluble polymeric particles in the lungs.

10. The interest of the problem of surfactant inhalation is not clear. Normally, they are used in solution. Are the authors discussing the inhalation of the powder surfactant? Otherwise the interest of the work is scarce. This point should be clarified.
Response: The majority of commercial surfactants are liquids and those that are powders are typically engineered to be of a non-respirable particle size. While we agree there is not significant consumer exposure via inhalation, surfactants are both manufactured and processed in industrial settings where exposure could be relevant. Additionally, there could be workplace exposure in commercial applications. EPA has the responsibility to assess these uses, which this manuscript addresses.

11. Authors mentions the subcategories of surfactants. However, they do not comment anything on the polymeric surfactant and the possible role of colloidal particles as surfactants.
Response: The most common classifications of surfactants are based on charge. These are nonionic, anionic, cationic, and amphoteric. Polymeric surfactants are included within each of these categories based on their charge or lack thereof. Most polymeric surfactants are nonionic. As an example, the nonionic surfactant Triton X-100 (ethoxylated octylphenol) is a polymeric surfactant.

This manuscript is focused on US EPA guidance related to the review of new chemical substances which are surfactants. Hence, colloidal particles are out of scope for this paper as they are not within the boundary criteria, nor would a colloidal suspension (e.g., a product containing particles suspended in another chemical) be subject to new chemical review.

Commented [HT7]: Discuss with Todd

12. Authors mention several test to evaluate the toxicity. However, they do not provide details on the foundation of such tests, making difficult the comparison of data.
Response: In the manuscript section entitled, **Use of New Approach Methods (NAMS) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing,** the authors first provide a summary table (Table 4) of potential methods for evaluating chemicals in the surfactant category. This summary table is then followed by descriptions of the scientific tenets of each of the tests referenced (e.g., On page X, "Sorli et al., (2017) developed an *in vitro* lung surfactant interaction assay that specifically measures whether a substance alters the surface tension of pulmonary surfactant." and on page Y, "...several human organotypic airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems." Additionally, specific details regarding some of the most recent or novel tests are also provided (e.g., on page X, "Organotypic airway cultures, such as EpiAirway™ and MucilAir™, take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain homeostasis for months in culture. All of the tests included in the summary table and the text have been published, either as journal articles or as test guidelines by international authorities and are fully referenced. To include additional methodological details about every referenced test would be unusual and would also be contrary to the reviewers' requests for shortening the manuscript.

Commented [SM8]: Agree

Reviewer: 2

Comments:
Henry et al investigated the development of a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. The paper describes in detail the literature found on surfactants and inhalation, suggest how AOPs and NAMs can be used for risk assessment, and a scheme for how this would done.

1. **[LENGTH]** The paper is well laid out, contains clear language and flows nicely. It describes very well how the risk assessment could be performed. However, I think the length could deter some readers, if the length is kept, I would suggest that you start with an index so that readers can find any information that they are most interested in.
   Response: The authors thank the reviewer for the complements on writing and flow. In response to the reviewer's comment regarding length of the manuscript, the authors have streamlined the paper significantly [XX pages and moved certain sections (e.g., YYY) into supplementary materials.

2. **[ABBREVIATIONS]** In line with this, the paper is very abbreviation heavy, so a list of abbreviations would be very helpful, this would also let you catch those abbreviations that are not defined (at least I could not find them). These include: BMCL (p.17) UFH, UFA, and UFL (p. 68).
   Response: The authors thank the reviewer for the suggestion to make a list of abbreviations; it was most helpful in conducting final review of the manuscript. The authors suggest including the list in supplementary materials due to concerns about the length of the manuscript but will defer to the Editor regarding final placement of the list. [Tala or Keith]

   Commented [SM9]: Abbreviation list might be help, reading our manuscript I had also often the feeling that we use a lot of abbreviation and they are not always present for the reader.

3. **[FONT]** It may be a product of conversion to pdf, but the font and size changes throughout the paper.
   Response: The font type and size has been checked and made consistent throughout the document. [Tala – Final Review]

4. **[REFERENCE FORMAT]** For the references section: Several references are inconsistent in order, as they are "Surname, intials, initals, surname"
   Response: All references have been checked and comply with the format requested by the journal. [Todd/Endnote]

   e.g. 54. Alarie, Y. and M.F. Stock, Respiratory Irritancy on a Mixture containing Polyethylene Glycol Mono(Octyl)Phenyl Eether CAS #9035-19-5. ChemView - U.S. Environmental Protection Agency, 1992: p. 37,
   Response: [EPA will do]

   - Also referencing to dossiers is strange (what is the R?):
   59. Dossier, R., N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Skin irritation/corrosion. European Chemicals Agency, 2020:
   - The links in references 89 and 90 do not work (I tried 2 different browsers, at separate days)
   Response: [EPA will do]

   Commented [SM10]: Strange, I could also not found the reference when searching direct on Echa page... also not the EC, but indirectly via google works, maybe there is a search problem on the ECHA website

   What about adding a unique number to the reference? The registration number is a number will stay the same over years.

5. **For page 29**: The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less.
   How is this tested?
   Response: [PAGE 18 of WORD document: add (as determined by standard methods)
   There are a number of techniques that utilize light scattering or steady-state fluorescence quenching to determine the formation of micelles and vesicles. The critical micelle concentration (CMC) value can be determined by plotting a curve of concentration versus surface tension. Since there are a variety of methods available, the authors did not want to suggest a specific method in order to provide flexibility to the reader.

6. **For page 75:** You write "including validated OECD methods for in vitro irritation testing and in vitro methods to specifically assess respiratory toxicity". This is not referenced, to my knowledge there are no validated OECD methods to specifically assess respiratory toxicity, please provide more info/reference.
   [redacted] [EPA will do]

7. **For page 54:** Cationic Surfactants
   You may like to include the paper "Airway Effects of Inhaled Quaternary Ammonium Compounds in Mice" by Larsen et al that describes airway effects after inhalation. Doi: 10.1111/j.1742-7843.2011.00851.x [ACUTE inhalation study of 4 Quats; relative potency in causing decreased tidal volume and increased respiratory rate indicating pulmonary irritation; pulmonary inflammation apparent from BAL; ADD TO IN VIVO SECTION OF CATIONIC – STARTS PAGE 34 OF WORD Document]
   Response: The authors have added a summary and refence to the study by Larsen et al. to the Hazard Identification section on Cationic Surfactants. Thank you for the citation. [Keith]

8. **For Scheme 1:** [Page 63 of WORD document]
   a. What does "CLEs" after Tier 2 stand for?
      Response: CLEs means Cellular Level Effect corresponding to the Level of Biological Organization in Table 4. The authors have included the abbreviation in Table 4 (first occurrence) and have added a legend to Scheme 1 in the revised manuscript.

      Commented [SM11]: event

   b. Also "OLEs", (presumably occupational exposure limits) is not defined in the text.
      Response: OLEs means Tissue or Organ Level Effect corresponding to the Level of Biological Organization in Table 4. The authors have included the abbreviation in Table 4 (first occurrence) and have added a legend to Scheme 1 in the revised manuscript.

      Commented [SM12]: see above

   c. Is the comparator substance the same in tier 2 and tier 3, if so how do the tiers differ?
      Response: [comparator = analog] Determination of the comparator would need to be considered at each Tier. Optimally, it would be best in compiling a weight of evidence that the comparator is the same across the testing tiers; however, there could be technical/testing issues that would make it necessary to use different.

      Commented [HT13]: Review for clarity/grammar

   d. What is the difference between "evidence does not indicate perturbation of AEC or PU surfactant" and "risk assessment complete"?
      Response: If the result of the decision criteria are not met then the evidence does not indicate perturbation of alveolar epithelial cells (AEC) or pulmonary (PU) surfactant. The authors have spelled these out rather than use acronyms in the revised manuscript.

OR
Revise this box in the Scheme to more clearly indicate the decision criteria not met means the chemical does not fit into the Category (for Tier 0 and Tier I) or lung toxicity is not a concern (Tier II and Tier III) …be more explicit about the hazard conclusion? [Todd or master of the Scheme]

Risk Assessment Complete indicates that if the if the testing conducted as designated in that Tier provides a point of departure (POD) for the toxic effect that can be used to quantify risk and the risk is acceptable under the regulatory scheme, then the risk assessment can be completed without conducting additional testing in higher tiers.

e. Would the former trigger testing so that you can achieve "risk assessment complete"
Response: Yes; if the if the testing conducted as designated in that Tier provides a point of departure (POD) for the toxic effect that can be used to quantify risk and the risk is acceptable under the regulatory scheme, then the risk assessment can be completed without conducting additional testing in higher tiers.

Commented [TH14]: All comment on Scheme language/clarity by COB Thursday 11/19/20

Some errors:

9. **Page 46:** missing space "Polysorbate 80 (Tween 80)and"
Response: The typographical error has been corrected.

10. **Page 60:** First line, a full stop too much "and. Ulceration"
Response: The typographical error has been corrected.

Message

From: Schweer, Greg [Schweer.Greg@epa.gov]
Sent: 2/26/2019 1:27:16 PM
To: Franz, Christina [Christina_Franz@americanchemistry.com]
CC: Pierce, Alison [Pierce.Alison@epa.gov]; Scheifele, Hans [Scheifele.Hans@epa.gov]; Henry, Tala [Henry.Tala@epa.gov]; Wormell, Lance [Wormell.Lance@epa.gov]; Starr, Richard [Richard_Starr@americanchemistry.com]; Brozena, Sarah [Sarah_Brozena@americanchemistry.com]; Blanco, Susan [Susan_Blanco@americanchemistry.com]; Blair, Susanna [Blair.Susanna@epa.gov]
Subject: RE: Names for the PMN Session
Attachments: bio-gallagher.docx; bio-prothero.docx; bio-schweer.docx; Gallagher.jpg; Prothero.jpg; Schweer.jpg

Christina,

Attached are the bios and pics for three of us (Greg Schweer, Jeff Gallagher, and Scott Prothero). I believe that Rebecca Edelstein already sent her bio and pic to you. I am awaiting bios/pics from Keith Salazar and David Tobias.

Our six presentations are currently being reviewed by the OCSPP IO. Hopefully, that review will be completed soon.

I received a registration confirmation from ACC Meeting Services. I assume that the other five EPA folks for this session will also be registered. Correct assumption?

From: Schweer, Greg
Sent: Monday, February 25, 2019 4:24 PM
To: 'Franz, Christina' <Christina_Franz@americanchemistry.com>
Cc: Pierce, Alison <Pierce.Alison@epa.gov>; Scheifele, Hans <Scheifele.Hans@epa.gov>; Henry, Tala <Henry.Tala@epa.gov>; Wormell, Lance <Wormell.Lance@epa.gov>; Starr, Richard <Richard_Starr@americanchemistry.com>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
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Subject: RE: Names for the PMN Session

We'll make it work.

Can everyone who has not done so already please send a brief bio and headshot to me as soon as possible?

Thanks very much.

Christina Franz

Senior Director, Regulatory & Technical Affairs
American Chemistry Council
700 Second St., NE
Washington, D.C. 20002
202-249-6406 (o)
Ex. 6 Personal Privacy (PP) - personal phone
Christina_Franz@americanchemistry.com

From: Schweer, Greg [mailto:Schweer.Greg@epa.gov]
Sent: Wednesday, February 20, 2019 4:04 PM
To: Franz, Christina <Christina_Franz@americanchemistry.com>
Cc: Pierce, Alison <Pierce.Alison@epa.gov>; Scheifele, Hans <Scheifele.Hans@epa.gov>; Henry, Tala <Henry.Tala@epa.gov>; Wormell, Lance <Wormell.Lance@epa.gov>; Starr, Richard <Richard_Starr@americanchemistry.com>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
Subject: Re: Names for the PMN Session

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Nonetheless, I just spoke with Tala Henry. All of our six presentations have been drafted and are being reviewed by OGC. We think that we can work still use all six EPA presenters but limit their presentations to 12 minutes each, on average, and thus ensure that Kelly has the 25 minutes you have allocated in the proposal below. In the first session, I will lead off, followed by Scott Prothero (worker exposure and environmental release assessment), David Tobias (fate, general population exposure, and consumer exposure), and Jeff Gallagher (eco hazard & risk). Keith Salazar (human health hazard & risk) will lead off the 2nd session, followed by Rebecca Edelstein, and then Kelly.

Sound OK to you?

From: Franz, Christina <Christina_Franz@americanchemistry.com>
Sent: Wednesday, February 20, 2019 1:22 PM
To: Schweer, Greg

Cc: Pierce, Alison; Scheifele, Hans; Henry, Tala; Wormell, Lance; Starr, Richard; Brozena, Sarah
Subject: RE: Names for the PMN Session

This was the proposal Kelly and I discussed:

9:30 AM - 10:30 AM PMN Workshop: Session 1
Greg Schweer, EPA (25 mins) – TSCA Section 5 Updates, new regulatory determinations, and introduction to the new chemical review process
David Tobias, EPA (25 mins) – Introduction of the Points to Consider Document and outline of additional information to provide with new chemical submissions
Q & A – 10 minutes

10:45 AM - 11:45 AM PMN Workshop: Session 2
Rebecca Edelstien, EPA (25 minutes) – PreNotice Communication process and how to coordinate with the Agency post-notification
Kelly Mayo, knoell USA (25 minutes) – Tips for preparing notification packages and strategies for supply chain communication
Q & A – 10 minutes

From: Franz, Christina
Sent: Wednesday, February 20, 2019 12:53 PM
To: Schweer, Greg
Cc: Pierce, Alison; Scheifele, Hans; Henry, Tala; Wormell, Lance; Starr, Richard; Brozena, Sarah
Subject: RE: Names for the PMN Session

Hello Greg:

I am not quite sure what happened in the communications between Richard Starr, Alison Pierce, Hans Scheifele and/or communications between Alison, Hans, and you, but let me try at least to explain my perspective on my own confusion and how I would propose to resolve the confusion.

The panel description that I was given on the Global Chem agenda identifies you and two other EPA presenters--David Tobias and Rebecca Edelstein. The fourth presenter was Kelly Mayo Bean. Kelly's organization is a sponsor, which is expensive and afforded her the opportunity to cover about 40 minutes of the presentation. That is why when I sent out the meeting invite for our discussion a few weeks ago, I sent it to you, David, Rebecca, and Kelly. I was very surprised when we had the call that you had as many people from EPA on the phone and all had a planned presentation. However, I didn't feel I was in a position to say much about it at the moment as a stand-in as moderator because I wasn't certain I had all the facts at my disposal. However, I was surprised by your comment, Greg, during the call that you did not know Kelly was a part of the panel.

Once our call was completed, it became clear the Kelly would not really have much of an opportunity to speak. This is posing a problem for her company as a sponsor. It appears to me, albeit from the outside since I am not part of the organizing team, that a possible miscommunication happened at EPA--largely because Greg said that he did not know Kelly was supposed to be a part of the panel. If I am incorrect about that, my apologies, I am only trying to piece this together. I think the only right thing to do is to return to the original format and divide the presentation up accordingly.

I had a phone conversation with Kelly and made notes on what that division might look like, but I am not in the office this week and do not have it in front of me. I would have to write to her and get that breakout by email. Perhaps it is readily apparent to you knowing her background and those of David and Rebecca, but it is not to me--apologies for that. I do recall that Kelly suggested perhaps the others from EPA you wanted to present could be in attendance to answer questions that the audience might have regardig their areas of expertise.

Does this make sense to folks?

I have copied Richard Starr and Sarah Brozena on this email as well--Richard because he is ACC's Global Chem organizer and Sarah because she was on our panel call with me, although we had agreed that I would moderate the panel.

In closing, it is a shame there has been a mixup with this session. I certainly don't want to offend anyone or cast any aspersions--these things happen sometimes. I just think returning to the original plan is the correct thing to do for all concerned.

Christina

From: Schweer, Greg [Schweer.Greg@epa.gov]
Sent: Wednesday, February 20, 2019 11:29 AM
To: Franz, Christina
Cc: Pierce, Alison; Scheifele, Hans; Henry, Tala; Wormell, Lance
Subject: Re: Names for the PMN Session

Christina,

This is an interesting turn of events. How do you and Kelly want to schedule her 40 minutes? We will need to shorten our presentations (or at least skip slides) or shorten the Q&A time.

From: Starr, Richard <Richard_Starr@americanchemistry.com>
Sent: Wednesday, February 20, 2019 10:55 AM
To: Scheifele, Hans
Cc: Pierce, Alison; Franz, Christina; Schweer, Greg
Subject: Re: Names for the PMN Session

Hi Hans, thanks for the note - I hope you're enjoying the light snow today. This matter is probably better resolved over the phone, but the weather is giving us little choice.

As happens sometimes, I think there may have been a mix up at some point. Kelly is with Knoell consulting, and they have paid for a sponsorship which encompasses a 40 minute presentation, so we will provide her that time, whether it is 20 minutes at the end of each half of the session (which may have been a source of the misunderstanding), or all at once. That is up to the group to split up, of course.

As I mentioned on the phone, the total time is 120 minutes (two one hour sessions with a 15 minute break in between), so the remaining 80 minutes is free to be split up however the group decides.

Sent from my iPhone

On Feb 19, 2019, at 10:06 PM, Scheifele, Hans <Scheifele.Hans@epa.gov> wrote:

> Hi Richard,

I'm following up on our conversation and email last Friday. Sorry for not getting back to you until now. I spoke with Greg Schweer and understand that he, Christina and others, including Kelly I believe, discussed the details last Wednesday and it was agreed that Kelly would have 20 minutes (at the end of the session as I understand it). I need to defer to the decisions of the planning group as discussed and agreed to last week. I've cc'ed Greg here if you have more questions. If I've misunderstood something Greg can clarify and/or discuss details further with Christina and you.

Thanks,
Hans

On Feb 14, 2019, at 1:36 PM, Starr, Richard <Richard_Starr@americanchemistry.com> wrote:

> Hi Alison (and Hans),
>
> Thank you for providing me with these names.
>
> We truly appreciate the interest in this session, though we'd like to ensure that the format leaves room for the non-EPA presenter on the panel.
>
> I'd like to reduce the number of formal presentations per hour to the original format (unless staff would like to tag in/out). This would ensure that each speaker/presenter could average about 20 minutes per hour including questions (Kelly Mayo's presentation is about 40 minutes, and would thus count for two slots). Any additional folks that do not make formal presentations could certainly be made available for Q&A portions of the session.
>
> I've copied Christina Franz, our moderator for the session, to clarify anything I missed, and help answer any questions about the format we're looking for.
>
> Thank you!
>
> Richard Starr | American Chemistry Council
> Manager, Regulatory & Technical Affairs
> richard_starr@americanchemistry.com
> 700 2nd Street, NE | Washington, DC | 20002
> O: (202) 249-6443 C: Ex. 6 Personal Privacy (PP) - personal phone
> www.americanchemistry.com
>
> ---
>
> **From:** Pierce, Alison [mailto:Pierce.Alison@epa.gov]
> **Sent:** Wednesday, February 13, 2019 3:12 PM
> **To:** Starr, Richard
> **Subject:** Names for the PMN Session
>
> Richard – Per discussion, here's our current slew of folks who will be helping out on the PMN session:
>
> - Greg Schweer

- Rebecca Edelstein
- David Tobias
- Jeff Gallagher
- Keith Salazar
- Scott Prothero

Best,
Alison

ALISON PIERCE
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460 USA

PIERCE.ALISON@EPA.GOV
202.564.2437

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Message

From: Schweer, Greg [Schweer.Greg@epa.gov]
Sent: 2/25/2019 9:23:59 PM
To: Franz, Christina [Christina_Franz@americanchemistry.com]
CC: Pierce, Alison [Pierce.Alison@epa.gov]; Scheifele, Hans [Scheifele.Hans@epa.gov]; Henry, Tala [Henry.Tala@epa.gov]; Wormell, Lance [Wormell.Lance@epa.gov]; Starr, Richard [Richard_Starr@americanchemistry.com]; Brozena, Sarah [Sarah_Brozena@americanchemistry.com]
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Ex. 6 Personal Privacy (PP) - personal phone
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Richard Starr | American Chemistry Council
Manager, Regulatory & Technical Affairs
richard_starr@americanchemistry.com
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O: (202) 249-6443 C: Ex. 6 Personal Privacy (PP) - personal phone
www.americanchemistry.com

From: Pierce, Alison [mailto:Pierce.Alison@epa.gov]
Sent: Wednesday, February 13, 2019 3:12 PM
To: Starr, Richard
Subject: Names for the PMN Session

Richard – Per discussion, here's our current slew of folks who will be helping out on the PMN session:

- Greg Schweer
- Rebecca Edelstein
- David Tobias
- Jeff Gallagher
- Keith Salazar
- Scott Prothero

Best,
Alison

ALISON PIERCE
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., N.W.
Washington, DC 20460 USA

PIERCE.ALISON@EPA.GOV
202.564.2437

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Message

From: Becker, Rick [Rick_Becker@americanchemistry.com]
Sent: 12/17/2020 2:04:42 PM
To: Stedeford, Todd [Stedeford.Todd@epa.gov]; American Chemistry Council LRI [ACC_LRI@icf.com]; amyjc@peta.org; rzaleski10@gmail.com; Henry, Tala [Henry.Tala@epa.gov]; Tan, Cecilia [Tan.Cecilia@epa.gov]; Patlewicz, Grace [Patlewicz.Grace@epa.gov]; ksullivan@pcrm.org; Vaughan-Dellarco, Vicki L. [dellarcov@gmail.com]; Barter, Robert A. [robert.a.barter@exxonmobil.com]; membry@hesiglobal.org; Jon Arnot [jon@arnotresearch.com]; Wambaugh, John [Wambaugh.John@epa.gov]; Scarano, Louis [Scarano.Louis@epa.gov]; Hartigan, Suzanne [Suzanne_Hartigan@americanchemistry.com]; Franz, Christina [Christina_Franz@americanchemistry.com]; Felter, Susan [Felter.sp@pg.com]; Ellison, Corie A. [ellison.ca@pg.com]; Rose, Jane [rose.jl@pg.com]; Salazar, Keith [Salazar.Keith@epa.gov]; McMahon, Tim [McMahon.Tim@epa.gov]; Rick_Becker@americanchemistry.com
Subject: Summary of WebEx and next steps re: developing the MS "Exploring Opportunities for Using the TTC in TSCA"
Attachments: TTC in TSCA_Draft Meeting Minutes_12_16_2020.docx

Hi everyone, and thanks to all of you who able to participate on the WebEx yesterday that focused on our developing the MS "Exploring Opportunities for Using the TTC in TSCA". Sorry the scheduling of this WebEx didn't allow us to include everyone. But that's OK. We are making good progress. In reviewing the edits and additions in the various sections, about half of you have had the opportunity to do some editing.

On the WebEx yesterday, a number of you indicated you plan to put some time into this over the next couple of weeks. With this in mind, here's a summary of the action items we discussed and agreed to with important target dates highlighted:

1. Please finish reviewing and editing the individual sections you've volunteered to work on no later than December 30th, 2020.

2. If you think of additional areas that should be included in the MS, please add these ideas to the "parking lot" section at the end of the "Draft Author by Section" document.

3. If you would like to volunteer to draft text pertaining to one of the ideas in the "parking lot" please put your name next to that entry.

4. Between December 30th and January 6th, I'll take all of the individual sections and "stitch" the sections together into one draft MS. The order of the sections in the MS will be adjusted in accordance with our discussions on the WebEx yesterday. I'll also flesh out the references section and do some I'll do some light editing too.

5. When this is completed, I'll archive the individual section drafts and post the full draft MS on the Google Docs site.

6. Will then send out an e-mail alerting you that the full draft MS is ready for all authors to review. We ask that everyone to read, review and make suggested edits to the entire MS.

7. If possible, would like to have all your edits and suggestions on the full draft MS completed by COB on January 18th.

Will then revise the MS accordingly that week, and hopefully it will then be in shape to put through our respective institutional review processes. Still eying submission by the end of January or as close to that date as we can achieve.

Best,

Rick

Opportunities for Using TTC in TSCA
WebEx Meeting on 12/16/2020
DRAFT Minutes Prepared by Steven Black

Attendees:

Rick Becker (ACC) (Host)
Vicki Dellarco (EPA, retired)
Amy Clippinger (PETA)
Rose Zaleski (Lumina Consulting)
Grace Patlewicz (EPA)
Corie Ellison (P&G)
Jon Arnot (ARC)
Kristie Sullivan (Physician's Committee)
Suzanne Hartigan (ACC)
Michelle Embry (HESI)
Todd Stedeford (EPA)
John Wambaugh (EPA)
Keith Salazar (EPA)
Steven Black (ICF)

Link to Google Folder: [HYPERLINK "https://drive.google.com/drive/folders/13HDTZbyJycFLbTrlrKNO_by-I85Q8p6b?usp=sharing"]

Agenda:

1. Welcome & Introductions, Antitrust Reminder
2. Provide update on each section to identify current and future plans
3. Discuss the option to organize separate WebEx meetings for co-authors of specific sections.
4. Discuss any other action items
5. Discuss next check in date.
6. Recap of action Items and Next Steps
7. Adjourn

1. **Welcome and anti-trust reminder**
   a. R. Becker welcomed the participants and reviewed the anti-trust reminder.
   b. R. Becker reviewed that ACC is interested in alternative approach methods and suggests that the TTC is an area where this could be done.
   c. Note that this does not represent any of the authors' employers' views.
   d. Any individual can edit or comment on any section, regardless of where they are listed as a section author.
2. **Review of each section and current state of the draft**
   a. R. Becker reviewed each section briefly.
   b. Next step is to take what is currently written and commented, compile it into one document, and then all authors would need to review and comment.
      1. T. Stedeford is reviewing section 5 and 6 still, but has noted that seeing what other sections have discussed would help frame what needs to be discussed in their section. T. Stedeford noted that combining the sections would help solve this.
      2. The rest of the group agreed with this plan.
   c. **R. Becker will not be compiling the document until early January, any comments added until December 30th are welcome.**

d. T. Stedeford suggested moving section 5 to after sections 8&9.
e. **The group should add ideas for incorporation into the document into the last box located in the Draft Author by Section document.**
f. M. Embry asked if others are having issues with not trying to repeat information that they wrote for the special issue on this topic or other papers they have written.
    1. C. Ellison commented that he encountered this but tried to get around it by having C. Tan take the first pass on the section so that it would change the perspective from his own writing.
    2. The group decided that this should be reviewed to make sure that what is written here is not duplicated in the other papers.
    3. R. Becker noted that maybe focusing on the overarching points is better instead of getting into the same details that may have been in that paper. He noted that linking and/or referencing to the other papers is acceptable.

3. Any other business?

a. R. Becker stated that some of these suggestions in the "parking lot" section may be assigned out to specific people.
b. T. Stedeford asked about the manuscript timeline.
    1. R. Becker stated that the timeline is extended to the end of January for the special issue.
        1. **Need the rough draft out in the first week of January, spend a week reviewing it, and then can get it out for clearance and for submission by the end of January.**

4. Recap of action items

a. All
    1. Finish reviewing individual sections no later than December 30th, 2020.
    2. Add ideas to the "parking lot" section at the end of the "Draft Author by Section" document.
    3. Review individual sections to ensure that there is as little overlap as possible between this manuscript and others in the special issue.
    4. Final review by January 15th.
b. Rick Becker
    1. Review and "stitch" the sections together into one draft document by Jan 8th, 2021.
    2. Follow up on ideas in the "parking lot" section and potentially assign out to topic experts.
    3. Oversee clearance and submission of manuscript.

5. Schedule next check-in meeting

ICF to poll when needed.

Message

From: Franz, Christina [Christina_Franz@americanchemistry.com]
Sent: 2/25/2019 8:25:53 PM
To: Schweer, Greg [Schweer.Greg@epa.gov]
CC: Pierce, Alison [Pierce.Alison@epa.gov]; Scheifele, Hans [Scheifele.Hans@epa.gov]; Henry, Tala [Henry.Tala@epa.gov]; Wormell, Lance [Wormell.Lance@epa.gov]; Starr, Richard [Richard_Starr@americanchemistry.com]; Brozena, Sarah [Sarah_Brozena@americanchemistry.com]
Subject: RE: Names for the PMN Session

We'll make it work.

Can everyone who has not done so already please send a brief bio and headshot to me as soon as possible?

Thanks very much.

Christina Franz
Senior Director, Regulatory & Technical Affairs
American Chemistry Council
700 Second St., NE
Washington, D.C. 20002
202-249-6406 (o)
Ex. 6 Personal Privacy (PP) - personal phone
Christina_Franz@americanchemistry.com

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To: Franz, Christina <Christina_Franz@americanchemistry.com>
Cc: Pierce, Alison <Pierce.Alison@epa.gov>; Scheifele, Hans <Scheifele.Hans@epa.gov>; Henry, Tala <Henry.Tala@epa.gov>; Wormell, Lance <Wormell.Lance@epa.gov>; Starr, Richard <Richard_Starr@americanchemistry.com>; Brozena, Sarah <Sarah_Brozena@americanchemistry.com>
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Christina,

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Nonetheless, I just spoke with Tala Henry. All of our six presentations have been drafted and are being reviewed by OGC. We think that we can work still use all six EPA presenters but limit their presentations to 12 minutes each, on average, and thus ensure that Kelly has the 25 minutes you have allocated in the proposal below. In the first session, I will lead off, followed by Scott Prothero (worker exposure and environmental release assessment), David Tobias (fate, general population exposure, and consumer exposure), and Jeff

Gallagher (eco hazard & risk). Keith Salazar (human health hazard & risk) will lead off the 2nd session, followed by Rebecca Edelstein, and then Kelly.

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Sent: Wednesday, February 20, 2019 1:22 PM
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Cc: Pierce, Alison; Scheifele, Hans; Henry, Tala; Wormell, Lance; Starr, Richard; Brozena, Sarah
Subject: RE: Names for the PMN Session

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Greg Schweer, EPA (25 mins) – TSCA Section 5 Updates, new regulatory determinations, and introduction to the new chemical review process
David Tobias, EPA (25 mins) – Introduction of the Points to Consider Document and outline of additional information to provide with new chemical submissions
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Subject: RE: Names for the PMN Session

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organizing team, that a possible miscommunication happened at EPA--largely because Greg said that he did not know Kelly was supposed to be a part of the panel. If I am incorrect about that, my apologies, I am only trying to piece this together. I think the only right thing to do is to return to the original format and divide the presentation up accordingly.

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Does this make sense to folks?

I have copied Richard Starr and Sarah Brozena on this email as well--Richard because he is ACC's Global Chem organizer and Sarah because she was on our panel call with me, although we had agreed that I would moderate the panel.

In closing, it is a shame there has been a mixup with this session. I certainly don't want to offend anyone or cast any aspersions--these things happen sometimes. I just think returning to the original plan is the correct thing to do for all concerned.

Christina

From: Schweer, Greg [Schweer.Greg@epa.gov]
Sent: Wednesday, February 20, 2019 11:29 AM
To: Franz, Christina
Cc: Pierce, Alison; Scheifele, Hans; Henry, Tala; Wormell, Lance
Subject: Re: Names for the PMN Session

Christina,

This is an interesting turn of events. How do you and Kelly want to schedule her 40 minutes? We will need to shorten our presentations (or at least skip slides) or shorten the Q&A time.

From: Starr, Richard <Richard_Starr@americanchemistry.com>
Sent: Wednesday, February 20, 2019 10:55 AM
To: Scheifele, Hans
Cc: Pierce, Alison; Franz, Christina; Schweer, Greg
Subject: Re: Names for the PMN Session

Hi Hans, thanks for the note - I hope you're enjoying the light snow today. This matter is probably better resolved over the phone, but the weather is giving us little choice.

As happens sometimes, I think there may have been a mix up at some point. Kelly is with Knoell consulting, and they have paid for a sponsorship which encompasses a 40 minute presentation, so we will provide her that time, whether it is 20 minutes at the end of each half of the session (which may have been a source of the misunderstanding), or all at once. That is up to the group to split up, of course.

As I mentioned on the phone, the total time is 120 minutes (two one hour sessions with a 15 minute break in between), so the remaining 80 minutes is free to be split up however the group decides.

Sent from my iPhone

On Feb 19, 2019, at 10:06 PM, Scheifele, Hans <Scheifele.Hans@epa.gov> wrote:

> Hi Richard,
>
> I'm following up on our conversation and email last Friday. Sorry for not getting back to you until now. I spoke with Greg Schweer and understand that he, Christina and others, including Kelly I believe, discussed the details last Wednesday and it was agreed that Kelly would have 20 minutes (at the end of the session as I understand it). I need to defer to the decisions of the planning group as discussed and agreed to last week. I've cc'ed Greg here if you have more questions. If I've misunderstood something Greg can clarify and/or discuss details further with Christina and you.
>
> Thanks,
> Hans
>
> On Feb 14, 2019, at 1:36 PM, Starr, Richard <Richard_Starr@americanchemistry.com> wrote:
>
> > Hi Alison (and Hans),
> >
> > Thank you for providing me with these names.
> >
> > We truly appreciate the interest in this session, though we'd like to ensure that the format leaves room for the non-EPA presenter on the panel.
> >
> > I'd like to reduce the number of formal presentations per hour to the original format (unless staff would like to tag in/out). This would ensure that each speaker/presenter could average about 20 minutes per hour including questions (Kelly Mayo's presentation is about 40 minutes, and would thus count for two slots). Any additional folks that do not make formal presentations could certainly be made available for Q&A portions of the session.
> >
> > I've copied Christina Franz, our moderator for the session, to clarify anything I missed, and help answer any questions about the format we're looking for.
> >
> > Thank you!
> >
> > Richard Starr | American Chemistry Council
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> Hi Richard,
>
> I'm following up on our conversation and email last Friday. Sorry for not getting back to you until now. I spoke with Greg Schweer and understand that he, Christina and others, including Kelly I believe, discussed the details last Wednesday and it was agreed that Kelly would have 20 minutes (at the end of the session as I understand it). I need to defer to the decisions of the planning group as discussed and agreed to last week. I've cc'ed Greg here if you have more questions. If I've misunderstood something Greg can clarify and/or discuss details further with Christina and you.
>
> Thanks,
> Hans
>
> On Feb 14, 2019, at 1:36 PM, Starr, Richard <Richard_Starr@americanchemistry.com> wrote:
>
> > Hi Alison (and Hans),
> >
> > Thank you for providing me with these names.
> >
> > We truly appreciate the interest in this session, though we'd like to ensure that the format leaves room for the non-EPA presenter on the panel.

I'd like to reduce the number of formal presentations per hour to the original format (unless staff would like to tag in/out). This would ensure that each speaker/presenter could average about 20 minutes per hour including questions (Kelly Mayo's presentation is about 40 minutes, and would thus count for two slots). Any additional folks that do not make formal presentations could certainly be made available for Q&A portions of the session.

I've copied Christina Franz, our moderator for the session, to clarify anything I missed, and help answer any questions about the format we're looking for.

Thank you!

Richard Starr | American Chemistry Council
Manager, Regulatory & Technical Affairs
richard_starr@americanchemistry.com
700 2nd Street, NE | Washington, DC | 20002
O: (202) 249-6443 C: Ex. 6 Personal Privacy (PP) - personal phone
www.americanchemistry.com

From: Pierce, Alison [mailto:Pierce.Alison@epa.gov]
Sent: Wednesday, February 13, 2019 3:12 PM
To: Starr, Richard
Subject: Names for the PMN Session

Richard – Per discussion, here's our current slew of folks who will be helping out on the PMN session:

- Greg Schweer
- Rebecca Edelstein
- David Tobias
- Jeff Gallagher
- Keith Salazar
- Scott Prothero

Best,
Alison

ALISON PIERCE
Office of Pollution Prevention and Toxics
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PIERCE.ALISON@EPA.GOV
202.564.2437

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Surfactants Category: The Application of a New Approach Methodology (NAM) for Assessing Inhalation Risks under the ~~Amended~~ Toxic Substances Control Act

Commented [A1]: Its just TSCA now

Tala R. Henry[a,‡], *Keith D. Salazar*[b,‡], *Michael P. Hayes*[c], *Wayne Kennedy*[d], *Athena M. Keene*[d], *Annie M. Jarabek*[e], *Stefan Moors*[f], *Lela Jovanovich*[g], *Jane L. Rose*[c], *Ann Tveit*[f], *Raphaël T. Tremblay*[c], *Richard A. Becker*[h], *Sahar Osman-Sypher*[h], *Patrick D. McMullen*[i], *Scott D. Slattery*[i], *William Irwin*[b], *Marc Odin*[j], *Julie Melia*[j], *Monita Sharma*[k], *Amy J. Clippinger*[k], *and Todd Stedeford*[a,*]

[a] Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC 20460, United States

[b] Risk Assessment Division, Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC 20460, United States

[c] Procter & Gamble, Company, Inc., St. Bernard, Ohio 45217, United States; Mason, Ohio 45040; Temselaan 100, 1853 Strombeek-Beaver, Belgium

[d] Afton Chemical Corporation, Richmond, Virginia 23219, United States

[e] Health & Environmental Effects Assessment Division, Center for Public Health & Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

[f] BASF Personal Care and Nutrition GmbH, Henkelstrasse 67, 40589 Duesseldorf, Germany; BASF Corporation, Florham Park, New Jersey 07932, United States

[g] Stepan Company, Northfield, Illinois 60093, United States

[h] American Chemistry Council, Washington, DC 20002, United States

[i] ScitoVation, Durham, North Carolina 27713, United States

[j] SRC, Inc., North Syracuse, New York 13212, United States

[k] PETA International Science Consortium Ltd., London, England

KEYWORDS: Inhalation, Surfactant, New Approach Methodologies, Lung Toxicity, Risk Assessment

ABSTRACT

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Abstract as Submitted = 359 Words//2,060Characters

Abstract as Revised = 293 Words//1,709 Characters

Surfactants are chemical substances used in a variety of industrial operations, occupational settings, and consumer products and therefore may result in exposure and toxicity in humans. The Toxic Substances Control Act (TSCA) requires anyone who plans to manufacture (including import) a new chemical substance for a ~~non-exempt commercial purpose~~ to provide the U.S. Environmental Protection Agency (EPA) with a premanufacture notice (PMN) prior to commercialization. ~~Surfactants are a class of chemical substances used in a variety of industrial operations, occupational settings, and in consumer products. Their uses in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to~~

~~humans. While TSCA requires submission of any existing toxicity data, it does not require generation of toxicity data for the purpose of, or prior to, submitting a PMN.~~ TSCA requires that EPA ~~to~~ review the PMN to determine whether the new chemical substance presents an unreasonable risk of injury to human health or the environment. While TSCA requires submission of existing toxicity data, it does not require generation of toxicity data to ~~for submitting~~ a PMN and it mandates that EPA reduce or replace vertebrate animals in testing, to the extent practicable and scientifically justified. EPA therefore relies on ~~several~~ approaches that do not rely on *de novo* toxicity testing to assess chemical risks, including ~~.~~ ~~A~~analogue read-across, in which toxicity data for a chemical of similar structure and activity is ~~are~~ used to assess the new chemical~~,~~ and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) ~~have been used by EPA for decades~~ to assess new chemical substances. This investigation establishes ~~was conducted to identify surfactant chemicals with toxicity data relevant for use in conducting a quantitative human health risk assessment for new surfactant substances and to define~~ a TSCA New Chemical Category for surfactants. ~~Category~~ The category described herein identifies physical-chemical properties to determine chemical inclusion/exclusion in the category, ~~boundaries, which are defined,~~ toxicological analogues suitable for ~~conducting~~ 'read-across' hazard assessment ~~(*i.e.*, hazard identification and dose-response analysis) are identified~~ and a tiered-testing strategy ~~aimed at~~ using new approach methodologies (NAMs) to reduce or replace animal testing ~~is outlined~~. This ~~tiered strategy to defining and evaluating the~~ Surfactant Category provides a pragmatic and scientifically defensible approach ~~to facilitate~~for EPA's review of PMNs for new surfactants and a strategic

testing approach to collect ~~hat provides the~~ data needed to ~~conduct or~~ refine surfactant risk assessments while also meeting the requirements of TSCA to reduce vertebrate testing.

INTRODUCTION

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The Toxic Substances Control Act (TSCA) was amended in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182). The amended TSCA included substantial changes to EPA's authorities and responsibilities, including requirements on EPA to make a determination regarding sufficiency of information, environmental releases and human exposure, and unreasonable risks. The amended TSCA also included provisions mandating EPA to "reduce and replace, to the extent practicable, [and] scientifically justified" the use of vertebrate animals in the testing of chemicals substances. Specifically, TSCA section 4(h) charges EPA with encouraging and facilitating –

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is any compound that reduces surface tension when dissolved in water or water solutions, or which reduces interfacial tension between two liquids, or between a liquid and a solid. [*Hawley's Condensed Chemical Dictionary, R. Lewis, Van Nostrand Reinhold Co.; 1993, pg. 1108.*] ~~a substance that reduces the surface tension of a liquid in which it is dissolved~~. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in industrial processes, occupational settings, and ~~in~~ consumer products (*e.g.*, household cleaning and ~~products,~~ personal care products, *etc.*) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The ~~widespread~~ manufacture, processing and use of surfactants provides opportunities for releases and exposure to humans ~~or environmental receptors~~. The inherent properties of surfactants may induce toxicity if exposures can interfere with biological surfactants or tissues. Certain surfactants are commonly used in a laboratory setting to disrupt cell membranes and denature proteins, which demonstrates the inherent hazards of surfactants. For example, sodium dodecyl sulfate (SDS; Chemical Abstracts Service Registry Number (CASRN) 151-21-3), a strong anionic surfactant, is used at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol (CASRN 9002-93-1), a mild nonionic surfactant, at concentrations up to 1% disrupt cell membranes, while preserving proteins for isolation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

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timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Hazard concerns for surfactants historically focused on their observed environmental effects and potential toxicity to aquatic organisms based on "down the drain" releases and/or presence in effluent from wastewater treatment facilities [ADDIN EN.CITE ADDIN EN.CITE.DATA].The EPA has established chemical categories for nonionic, anionic, and cationic (quaternary ammonium) surfactants based on environmental toxicity concerns [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-

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title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>]. Surfactants may pose a potential hazard to humans, depending on their use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. Mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" [ADDIN EN.CITE <EndNote><Cite><Author>Fox</Author><Year>2008</Year><RecNum>14730</RecNum><DisplayText>[4]</DisplayText><record><rec-number>14730</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017801">14730</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Fox, D.A.</author><author>Boyes, W.K.</author></authors><secondary-authors><author>Klaassen, C.D.</author></secondary-authors></contributors><titles><title>Toxic Responses of the Ocular and Visual System</title><secondary-title>Casarett & Doull's Toxicology - The Basic Science of Poisons, Seventh Edition</secondary-title></titles><pages>665-697</pages><section>17</section><dates><year>2008</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill, Medical Publishing Division</publisher><urls></urls></record></Cite></EndNote>].

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Depending on the conditions of use, the potential for inhalation exposures to workers and/or consumers warrant consideration in quantitative risk assessments. Surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and interfere with the natural pulmonary surfactants and result in reduction in the oxygen content of arterial blood due to impaired gas exchange in the pulmonary region, increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The chemical category boundary for surfactants that may have the potential to present an inhalation hazard has not been previously defined. The toxicity of surfactants by inhalation exposure can vary over several orders of magnitude, based on their chemical properties, although differences in exposure conditions are an important confounder to consider in cross category comparisons. For example, among the available data, a lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) was determined for octylphenoxypolyethoxyethanol, a nonionic surfactant, in a 14-day whole body study[ADDIN EN.CITE ADDIN EN.CITE.DATA] while a LOAEC of 0.08 mg/m^3 in a 4-week nose-only study [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington,

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D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] was observed for didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5), a cationic surfactant and biocide.

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The objectives of the present investigation were to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify inhalation toxicity studies on surfactants that may be used to inform inhalation risk assessments; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing; and (4) establish a tiered-testing strategy that uses NAMs to evaluate new chemistries in the Surfactant Category.

MATERIALS AND METHODS

Systematic Literature Review

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Much of the editing in the Supplemental

Two literature searches were performed, an initial search from 1950 through November 2016 and a supplemental search up to April 2018. The details of these searches, including the search strategies, search terms, search results and Population, Exposure, Comparison, and Outcome (PECO) criteria used for reviewing the relevance of the identified studies to this evaluation are provided in the Supporting Information file at "Section 1 Systematic Literature Review". These searches were conducted with the primary objective of identifying studies that evaluated the toxicity of surfactants in the respiratory tract of humans or laboratory animals, and at the cellular

level in *in vitro* and *ex vivo* studies. In addition, these searches were used to identify potential NAMs that could inform a tiered-testing strategy for general surfactants that reduces or replaces the use of vertebrate animals in regulatory testing.

Risk Assessment Approaches under TSCA

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Risk Assessment Paradigm

~~The methods for assessing~~Assessment of risks of new chemical substances under TSCA ~~have been developed using science-based approaches, scientific peer review, and refinement of the approaches. EPA conducts risk assessments~~ follows ~~ing~~ the four-step process articulated by the U.S. National Research Council (NRC) in 1983 [11] and reaffirmed several times since its initial release [12, 13]. This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the adverse health or environmental effects, or hazards, that can be caused by exposure to a chemical substance. The dose-response analysis assesses the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects. The exposure assessment characterizes human or environmental exposures, including the magnitude, frequency, and duration, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure components to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these risk assessment components, including the level of detail and complexity of quantitative aspects, may vary across different risk assessments and typically align

with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14738</RecNum><DisplayText>[11]</DisplayText><record><rec-number>14738</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019129">14738</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>40 CFR Part 720 - Premanufacture Notification</title><secondary-title>Code of Federal Regulations</secondary-title></titles><periodical><full-title>Code of Federal Regulations</full-title></periodical><pages>https://www.law.cornell.edu/cfr/text/40/part-720</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], companies are required to submit a PMN along with available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances.

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives hazard data for new chemical substances. An analysis of toxicity data submitted to EPA from 2004 through 2012 for new chemical substances found that only about 15% of the PMN submissions included health hazard data; the majority of which was that information was for acute toxicity ~~(e.g., 24-hour dermal toxicity study with a 14-day post-administration observation period)~~ and irritation ~~(e.g., 4-hour dermal irritation/corrosion with a 14-day post-administration observation period or 24-hour eye irritation/corrosion with a 21-day post-administration observation period)~~ in laboratory animals. TSCA provides EPA with the authority to require the generation ~~and submission~~ of additional data when the information included with the PMN ~~coupled with that available to EPA risk assessors from predictive modeling, read-across, internal archives, *etc.*~~ is insufficient to permit a reasoned evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must first take into consideration reasonably available existing information, including toxicity information (*e.g.*, in the scientific literature or internal archives, *etc.*; computational toxicology and bioinformatics (*e.g.*, predictive modeling, read-across); and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

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Given the historical lack of hazard data for new chemical substances, EPA has, for decades, employed a number of approaches that do not rely on *de novo* toxicity testing. These approaches

include computational toxicology (*e.g.*, predictive models and expert systems), analogue[1] read-across wherein available toxicity data for a chemical of similar structure and activity are used to assess the new chemical substance lacking data, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. EPA has a ~~'s current~~ chemical categories document on surfactants entitled "TSCA New Chemicals Program (NCP) Chemical Categories" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] that includes information for

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[1] In the context of this article, an analogue is a chemical substance identified based on its physicochemical and toxicological properties, as one that has undergone evaluation, as stated above, and determined to be an acceptable toxicological analogue for read across to the new chemical substance. An analogue may be directly used in read-across for informing a quantitative risk assessment on a new chemical substance.

anionic, nonionic, and cationic surfactants; however, ~~these were previously~~the categories are ~~developed and~~ defined only for ~~an~~ environmental toxicity considerations.

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The integration of these methods with NAMs to advance testing strategies has been recognized by Dellarco *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] and is consistent with the vision articulated in the 2007 report by the NRC in "Toxicity Testing in the 21st Century: A Vision and Strategy" [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[16]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>]. EPA defines NAMs "as a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14844</RecNum><DisplayText>[17]</DisplayText><record><rec-number>14844</rec-number><foreign-

keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1597332016">14844</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA Program</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>39, https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf</pages><volume>EPA-740-R1-8004</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]

Dose-Response Analysis

~~In the absence of test data on new chemical substances, EPA relies on read-across methods using an analogue or a category of analogues in the absence of test data on the new chemical substance to identify hazards and conduct d~~Dose-response analysis is conducted, ~~whether on a new chemical substance or an appropriate analogue,~~ to identify a point of departure (POD), *i.e.*, a dose or concentration that marks the beginning of a low-dose extrapolation. In the absence of test data on new chemical substances ~~T~~toxicity data for analogues are used to identify a POD, such as a no observed adverse effect (concentration) level (NOAE(C)L) or lowest observed adverse effect (concentration) level (LOAE(C)L, for assessing risks of the new chemical substance. This

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POD can also be the lower bound on dose (or concentration) for an estimated incidence or a change in response level calculated by a dose-response model such as those available in EPA's benchmark dose software (BMDS), *e.g.*, the BMCL for an observed incidence or change in level of response [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>14744</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14744</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019975">14744</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Benchmark Dose Technical Guidance</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>99, https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf</pages><volume>EPA/100/R-12/001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>]. ~~EPA's current chemical categories document on surfactants entitled "TSCA New Chemicals Program (NCP) Chemical Categories"~~[ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] ~~includes information for anionic, nonionic, and cationic surfactants; however, these were previously developed and defined only on environmental toxicity considerations.~~

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EPA's ~~has also developed~~ guidance to improve the science underlying the animal-to-human uncertainty factor (UF_H), which ~~and~~ provides generalized procedures for deriving dosimetric adjustment factors (DAFs) to perform interspecies extrapolation [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 20]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

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20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>] is also used in dose-response analysis. Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the human equivalent concentration (HEC). Application of a DAF in the calculation of an HEC is considered to address the toxicokinetic (TK; *i.e.*, absorption, distribution, metabolism, and excretion) aspects, but not the toxicodynamic (TD; *i.e.*, mode of

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action) component, of the ~~animal-to-human uncertainty factor~~ (UF_{H}) (*i.e.*, to estimate from animal exposure information the human exposure scenario that would result in the same dose as achieved in the animal to a given target tissue) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>]. This operational derivation of a DAF involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle, reactive gas, or volatile organic compound) coupled with consideration of the location and type of toxic response. These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (*e.g.*, to a weekly average based on number of h/d and d/w).

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For interspecies extrapolation of particle exposures, the Regional Deposited Dose Ratio (RDDR) model developed by EPA can be used to derive a DAF. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. EPA's RDDR model allows calculation of RDDR estimates in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic [ET], tracheobronchial [TB], pulmonary [PU], thoracic [TH], total respiratory tract [RT] and extra-respiratory [ER] regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (*i.e.*, the Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or

GSD, and density), and species-specific parameters for both animals and humans including ventilation rates and regional surface areas of the respiratory tract. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The DAF is then applied to the duration-adjusted POD to arrive at the HEC of the POD (POD_{HEC}). The EPA's RDDR model was used herein to calculate HEC values from the aerosol exposures to laboratory animals available for each of the surfactant classes.

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After an analogue(s) is identified, the strengths, limitations, and uncertainties associated with the use of the ~~substance~~analogue(s) to predict the hazards and POD for the new chemical substance under evaluation are considered when deriving a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant UFs to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL to a NOAEL [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 21]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A

Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. EPA prefers using existing information to develop data-derived extrapolation factors (DDEFs) or chemical specific adjustment factors (CSAFs) rather than relying on default values [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><DisplayText>[21]</DisplayText><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. This investigation includes several approaches to derive DDEFs for use in assessing new surfactant chemical substances.

Exposure Assessment

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In assessing new chemical substances, generally new chemical substances do not have occupational exposure monitoring data or consumer exposure data; therefore, EPA typically evaluates occupational exposures first, given that these represent the highest exposure estimates. Therefore, this evaluation focused on occupational exposures, recognizing that consumer exposures would also be considered, if applicable. EPA develops exposure estimates for workers using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER)

model. ChemSTEER estimates exposure as daily acute potential dose rates (PDRs) or lifetime average daily doses (LADDs). The PDR represents average exposure over an 8-hour workday, whereas the LADD estimates long-term exposures to the chemical substance and is averaged over a lifetime exposure of 75 years. The PDR, an initial conservative exposure estimate, is considered to be the more appropriate dose-metric for estimating risks to surfactants because surfactants are surface-active at the point of exposure and effects in the respiratory tract occur rapidly following exposure. This assumes that neither the chemical nor its damage accumulate or distribute to systemic compartments. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR values are 1.875 mg/kg-bw/day for inhalable aerosols or 0.625 mg/kg-bw/day for respirable aerosols as shown in [REF _Ref46930162 \h * MERGEFORMAT] [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2015</Year><RecNum>14745</RecNum><DisplayText>[22]</DisplayText><record><rec-number>14745</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021217">14745</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>ChemSTEER User Guide, Chemical Screening Tool for Exposures and Environmental Releases</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>403, https://www.epa.gov/sites/production/files/2015-

05/documents/user_guide.pdf</pages><dates><year>2015</year></dates><urls></urls></recor

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Table [SEQ Table * ARABIC]. Default values used for calculating the daily acute potential dose rate (PDR).

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Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg-bw	kg-bw

[a] Cm may also be adjusted for the mass concentration of the chemical with a permissible exposure limit (PEL) in air (based on the U.S. Occupational Safety and Health Administration [OSHA] PEL – time-weighted average [TWA]; where: KCk = the mass concentration limit of total particulate in air (mg/m^3) with a default of 15 mg/m^3 for inhalable and 5 mg/m^3 for respirable, Ys= the weight fraction of chemical in particulate ($0 < Ys \leq 1$), Ypel=the weight fraction of chemical or metal in particulate with a known PEL ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

The PDR is calculated using an exposure regimen for a default worker of 8 hours/day and 5 days/week, unless chemical-specific manufacture, processing or use information are provided in the PMN. The exposure conditions in laboratory animal studies often do not reflect occupational exposure scenarios; therefore, a duration adjustment and a DAF (*i.e.*, RDDR value) are applied to the POD to derive HECs for exposed human populations according to Agency methods [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Therefore, the interspecies extrapolation is performed using particle deposition models that adjust for the aerodynamics of the given particles in the different airway architecture between the

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species and using species-specific physiologic parameters such as ventilation. The occupational exposure is characterized with human ventilation rates during exertion (work) and exposure durations appropriate to the specific occupational setting and chemical use scenario.

Risk Characterization

~~Risk characterization is the final, integrative step of risk assessment.~~ EPA's Risk Characterization Policy defines risk characterization as the integration of information from the hazard and exposure components of the risk assessment into an overall conclusion about the existence (or lack of) ~~risk that is complete, informative, and useful for decision-making. The risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of)~~ human health or ecological risks [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2000</Year><RecNum>14747</RecNum><DisplayText>[23]</DisplayText><record><rec-number>14747</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021806">14747</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Risk Characterization</title><secondary-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>189, https://nepis.epa.gov/Exe/ZyPDF.cgi/40000006.PDF?Dockey=40000006.PDF</pages><volume

>EPA 100-B-00-

002</volume><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>].

It is recognized that ~~As described in EPA's Risk Characterization Handbook~~ "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized and the level of information contained in each risk characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios. The MOE is the HEC derived from a POD for a health endpoint (from hazard assessment) divided by the exposure concentration for the scenario of concern (from exposure assessment). The calculated MOE is compared with a benchmark MOE to evaluate whether there is an adequate margin between human exposure estimates and the HEC. When the MOE is less than the benchmark MOE, there is a possibility of human health risks. On the other hand, risks are not expected ~~negligible concerns would be expected~~ if the MOE exceeds the benchmark MOE. ~~The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios.~~

In summary, in developing a risk assessment for new chemical substances under TSCA section 5, EPA uses empirical data or analogues, to identify a POD(s) and to develop an exposure estimate for use in the evaluation. The hazard assessment in combination with the exposure assessment is used to calculate an MOE, which is compared to the benchmark MOE to identify potential risks. The risk characterization is used to inform the TSCA "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

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An initial search of PubMed identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles that met the PECO criteria were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc*., and were included for full text review. Of the 60 articles evaluated through full text screening, 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search of PubMed and Embase, 1247 articles (combined) were identified. Following title and abstract screening, 1217 of these articles were excluded because they did not meet the PECO criteria, whereas 25 met the PECO criteria and were selected for full text review. An additional 10 studies that met the PECO criteria were found by additional hand searching) and were selected for full text screening, which resulted in 35 articles that were

identified for review; ten articles were deemed irrelevant and excluded. A total of 25 articles were identified from both searches, one was excluded because it was in a foreign language and the remaining 24 articles are summarized in Table 8 in the Supporting Information file at "Section 1 Systematic Literature Review".

The information identified in the systematic review was used to determine Category Boundaries and subcategories, to summarize the health effects of surfactants under the section on Hazard Identification, and to identify potential NAMs for use in the Tiered-Testing Strategies.

Category Boundaries

The following structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) [ADDIN EN.CITE ADDIN EN.CITE.DATA]:

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance is capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test concentration of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less (as measured using a standard method).

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ADD A BRIEF DESCRIPTION/ PARENTHETICAL AND REFERENC HERE

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The Surfactants Category is further defined into three general subcategories including nonionic, anionic, and cationic substances. Amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, anionic and cationic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging from 6.6 to 7.1 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The pKa for each component of an amphoteric surfactant should be evaluated within this pH range and the assessment should be conducted on the predominant components. The non-ionized fraction for acids/bases is calculated as follows:

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pH-pKa}})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pKa-pH}})$$

Where the pH represents the physiological pH in the lung lining fluid (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine).

Nonionic surfactants are identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Examples of ~~For example, octylphenoxypolyethoxyethanol, a~~ common

nonionic surfactants and the range of corresponding surface tension measurements associated with them ~~octylphenol EO surfactant, and Polysorbate 80 (or Tween 80; CASRN: 9005-65-6), another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units,~~ are shown in [REF _Ref47613375 \h * MERGEFORMAT]. ~~The surface tensions of octylphenoxypolyethoxyethanol and Polysorbate 80 range from 30-31 mN/m to 37-96 mN/m, respectively ([~~ REF _Ref47613375 \h * MERGEFORMAT]~~)~~[ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware, A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].

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Anionic surfactants are identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates,

alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). An example anionic surfactant, SDS, has a reported surface tension of 35 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]).

Cationic surfactants are identified as any chemical substance with a net positive charge that meets the Surfactant Criteria ~~(e.g., alkylammonium chlorides and benzalkonium chlorides). Benzalkonium chloride (BAC; CASRN 8001-54-5) and didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5) are R~~representative members of this subcategory, with surface tensions ~~of 37 mN/m and 25.82 mN/m ([~~ REF _Ref47613375 \h * MERGEFORMAT]~~), respectively~~ are provided in Table 2. It is noted that BAC and DDAC also possess biocidal properties.

Typical commercial surfactants (nonionic, anionic, and cationic) are non-volatile[3] liquids or solids. This category framework focuses on exposure *via* aerosol forms (*i.e.*, both airborne droplets and solid particles, including the hygroscopic variety) of these surfactants. While the commercial use of volatile surfactants is unlikely, it should be noted that this framework is not applicable to any substances that qualify as surfactants and are volatile under the conditions of use.

[3] Volatility is considered as part of the ChemSTEER modeling, wherein a vapor pressure of 1.3×10^{-04} kPa is the cutoff for gases/vapors.

Table [SEQ Table * ARABIC]. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cationic Subcategorization.

Nonionic Surfactants					
Chemical Name in Text	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**
		Hydrophobic group(s)	**Hydrophilic group(s)**	**Surface Tension**	**Critical Micelle Concentration (CMC)**
formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol Defomaire Alevaire Tyloxapol CASRN: 25301-02-4	CAS Name: formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol	multiple octyl phenol groups	multiple polyoxyethylene (9) units	~37 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,	0.038 g/L or 0.0038 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sch

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				H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-	ott, H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface

				1></periodical><alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-database-provider>NLM</remote-database-	science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-

				provider><language>eng</language></record></Cite></EndNote>]	num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
octylphenoxypolyethoxyethanol CASRN: 9002-93-1	Triton X-100 Octoxynol 9 octylphenol ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	octylphenol group	polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott, H.</author></authors>	0.17 g/L or 0.017 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,

				</contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-	H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-

				periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>e	title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-

				ng</language></record></Cite></EndNote>]	database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$) CASRN: 9004-98-2	oleyl ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	oleyl group	polyoxyethylene (10) unit	35.17 mN/m at 4×10^{-5} M (0.028 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[32]</DisplayText><record><rec-number>14761</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,	4×10^{-5} M or 0.028 wt % at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[32]</DisplayText><record><rec-number>14761</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,

				F.</author><author>Wang, Z.</author><author>Sun, D.</author><author>Wei, X.</author><author>Zhou, W.</author><author>Li, G.</author><author>Zhang, G.</author></authors></contributors><titles><title>Adsorption Kinetics of Brij 97 at the Air/Solution Interface</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>657-663, https://www.tandfonline.com/doi/abs/10.1080/01932690600660624</pages><volume>27	F.</author><author>Wang, Z.</author><author>Sun, D.</author><author>Wei, X.</author><author>Zhou, W.</author><author>Li, G.</author><author>Zhang, G.</author></authors></contributors><titles><title>Adsorption Kinetics of Brij 97 at the Air/Solution Interface</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>657-663, https://www.tandfonline.com/doi/abs/10.10

				</volume><number>5</number><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]	80/019326906006606 24</pages><volume> 27</volume><numbe r>5</number><dates ><year>2006</year> </dates><urls></urls ></record></Cite></ EndNote>]
polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$) CASRN: 9002-92-0	polyoxyethylene (10) lauryl ether CAS Name: poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-	dodecyl group	polyoxyethylene (10) unit	C12E9: 36 mN/m (concentration not reported) at 23°C* C12E12: 32 mN/m (concentration not reported) at 23°C* [ADDIN EN.CITE <EndNote><Cite><Au thor>Rosen</Author> <Year>1989</Year>< RecNum>14763</Rec Num><DisplayText>[33]</DisplayText><re cord><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0 azr5evearxfds0err5sr" timestamp="15960265 43">14763</key></for eign-keys><ref-type name="Edited	12.7×10^{-6} M or 0.0008 wt% at 30°C [ADDIN EN.CITE <EndNote><Cite><A uthor>Sulthana</Aut hor><Year>2000</Y ear><RecNum>1476 2</RecNum><Displa yText>[34]</Display Text><record><rec-number>14762</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre 0azr5evearxfds0err5s r" timestamp="1596025 808">14762</key></ foreign-keys><ref-type name="Journal Article">17</ref-type><contributors>< authors><author>Sult hana,

				Book">28</ref-type><contributors><authors><author>Rosen, M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431, </pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]	S.B.</author><author>Rao, P.V.C.</author><author>Bhat, S.G.T.</author><author>Sugihara, N.G.</author><author>Rakshit, A.K.</author></authors></contributors><titles><title>Solution Properties of Nonionic Surfactants and Their Mixtures: Polyoxyethylene (10) Alkyl Ether [CnE10] and MEGA-10</title><secondary-title>Langmuir</secondary-title></titles><periodical><full-title>Langmuir : the ACS journal of surfaces and colloids</full-title><abbr-1>Langmuir</abbr-1></periodical><pages>980-987, https://doi.org/10.1021/la990730o</pages>

					<volume>16</volume><number>3</number><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>] Also, C12E9 at 1×10^{-6} M at 23°C and C12E12 at 1.4×10^{-6} M at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Rosen</Author><Year>1989</Year><RecNum>14763</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026543">14763</key></foreign-keys><ref-type name="Edited Book">28</ref-type><contributors><authors><author>Rosen,

					M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431, </pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]
Polysorbate 20 (Tween 20) CASRN: 9005-64-5	polyoxyethylene (20) sorbitan monolaurate CAS Name: sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	dodecanoyl group	sorbitan polyoxyethylene (20) unit	38 mN/m at 8.04×10^{-5} M (0.001 wt%) and 21°C* [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[35]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.04×10^{-5} M or 0.001 wt% at 21°C [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[35]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5s

				azr5evearxfds0err5sr" timestamp="1596024348">14756</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, C.</author><author>Hsieh, Y.-L.</author></authors></contributors><titles><title>Wetting and absorbency of nonionic surfactant solutions on cotton fabrics</title><secondary-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</secondary-title></titles><periodical><full-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</full-title></periodical><pages>385-397</pages><volume>187-188</volume><numbe	r" timestamp="1596024348">14756</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, C.</author><author>Hsieh, Y.-L.</author></authors></contributors><titles><title>Wetting and absorbency of nonionic surfactant solutions on cotton fabrics</title><secondary-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</secondary-title></titles><periodical><full-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</full-title></periodical><pages>385-397</pages><volume

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				r>31</number><dates><year>2001</year></dates><urls></urls></record></Cite></EndNote>]	>187-188</volume><number>31</number><dates><year>2001</year></dates><urls></urls></record></Cite></EndNote>]
Polysorbate 80 (Tween 80) CASRN: 9005-65-6	polyoxyethylene (20) sorbitan monooleate CAS Name: sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	octadecenoyl group	sorbitan polyoxyethylene (20) unit	37.96 mN/m at 5 g/L (0.5 wt%) and 30°C [ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware,	1.5×10^{-5} M or 0.002 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mahmood</Author><Year>2013</Year><RecNum>14757</RecNum><DisplayText>[36]</DisplayText><record><rec-number>14757</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024783">14757</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mahmood, M.E.</author><autho

				A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></reco	r>Al-Koofee, D.A.F.</author></authors></contributors><titles><title>Effect of Temperature Changes on Critical Micelle Concentration for Tween Series Surfactants</title><secondary-title>Global Journal of Science Frontier Research Chemistry</secondary-title></titles><periodical><full-title>Global Journal of Science Frontier Research Chemistry</full-title></periodical><pages>5, https://journalofscience.org/index.php/GJSFR/article/view/816/681</pages><volume>13(B)</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]

				rd></Cite></EndNote >]	
Poloxamer 188 CASRN: 691397-13-4	CAS Name: oxirane, 2-methyl-, polymer with oxirane, triblock	polyoxypropylene (27) unit	two polyoxyethylene (80) units	~42-44 mN/m at ~0.5 wt% and 36°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]	4.8×10^{-4} M or 0.4 wt% at 37°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]
N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$)*** CASRN: 1643-20-5	lauryl dimethylamine oxide CAS Name:1-dodecanamine, N,N-dimethyl-, N-oxide	dodecyl group	amine oxide unit	34.1 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14772</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14772</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596028055">14772</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></aut	1.7×10^{-3} M or 0.039 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Hoffmann</Author><Year>1990</Year><RecNum>14764</RecNum><DisplayText>[40]</DisplayText><record><rec-number>14764</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026736">14764</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hoffmann,

				hors></contributors><titles><title>Dodecyldimethylamine oxide, CASRN: 1643-20-5, EC number: 216-700-6, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-dossier/10062/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	H.</author></authors></contributors><titles><title>Correlation between surface and interfacial tensions with micellar structures and properties of surfactant solutions</title><secondary-title>Progress in Colloid & Polymer Science</secondary-title></titles><periodical><full-title>Progress in Colloid & Polymer Science</full-title></periodical><pages>16-28, https://link.springer.com/chapter/10.1007%2FBFb0116238</pages><volume>18</volume><dates><year>1990</year></dates><urls></urls></record></Cite></EndNote>] 1×10^{-5} M to 5.5×10^{-5} M or 0.0002 to 0.001

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					wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle concentrations of aqueous surfactant systems</title><seco

					ndary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
Anionic Surfactants					
Chemical	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**

Name in Text		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
sodium dodecyl sulfate (SDS) CASRN: 151-21-3	CAS Name: sulfuric acid monododecyl ester sodium salt (1:1)	dodecyl group	sulfate group	35 mN/m at 0.29 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Hernainz</Author><Year>2002</Year><RecNum>14768</RecNum><DisplayText>[42]</DisplayText><record><rec-number>14768</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027363">14768</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hernainz, F.</author><author>Caro, A.</author></authors></contributors><titles><title>Variation of surface tension in	8.25×10^{-3} M or 0.24 wt% at 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical

				aqueous solutions of sodium dodecyl sulfate in the flotation batch</title><secondary-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</secondary-title></titles><periodical><full-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</full-title></periodical><pages>19-24, https://www.sciencedirect.com/science/article/abs/pii/S0927775701005751</pages><volume>196</volume><number>1</number><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>]	micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]

oleoyl sarcosine CASRN: 110-25-8	CAS Name: glycine, N-methyl-N-((9Z)-1-oxo-9-octadecen-1-y	oleyl group	carboxylic acid anion	31.91 mN/m at 0.1 wt% and 19.9°C** [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14767</RecNum><DisplayText>[43]</DisplayText><record><rec-number>14767</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027202">14767</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-methyl-N-(1-oxo-9-octadecenyl)aminoacetate, CASRN 3624-77-9, EC number: 222-829-9, Surface Tension</title><secon	2.6×10^{-3} wt% and ~25°C ** (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[44]</DisplayText><record><rec-number>14769</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027596">14769</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ChattemChemicals</author></authors></contributors><titles><title>Oleoyl Sarcosine, CASRN 110-25-

				dary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://www.echa.europa.eu/fi/web/guest/registration-dossier/-/registered-dossier/5350/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	8</title><secondary-title>Product Information</secondary-title></titles><periodical><full-title>Product Information</full-title></periodical><pages>https://www.chattemchemicals.com/</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]
sodium lauroyl sarcosinate CASRN: 137-16-6	CAS Name: glycine, N-methyl-N-(1-oxododecyl)-, sodium salt (1:1)	lauryl group	carboxylic acid anion	40.5 mN/m at 2 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14770</RecNum><DisplayText>[45]</DisplayText><record><rec-number>14770</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.0×10^{-2} wt% and ~25°C (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[44]</DisplayText><record><rec-number>14769</rec-

				azr5evearxfds0err5sr" timestamp="1596027817">14770</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN 137-16-6, EC number: 205-281-5, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-dossier/14123/4/11</pages><dates><year>2020</year></dates><u	number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027596">14769</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ChattemChemicals</author></authors></contributors><titles><title>Oleoyl Sarcosine, CASRN 110-25-8</title><secondary-title>Product Information</secondary-title></titles><periodical><full-title>Product Information</full-title></periodical><pages>https://www.chattemchemicals.com/</pages><dates><year>2020</year></dates><urls></urls></rec

				rls></urls></record></Cite></EndNote>]	ord></Cite></EndNote>]
dioctyl sulfosuccinate sodium salt (DOSS) CASRN: 577-11-7	dioctyl sodium sulfosuccinate CAS Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt	two 2-ethyl hexyl groups	sulfosuccinate group	<28 mN/m at 0.5 vol% and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Williams</Author><Year>1957</Year><RecNum>14755</RecNum><DisplayText>[46]</DisplayText><record><rec-number>14755</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024180">14755</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Williams, E.F.</author><author>Woodberry, N.T.</author><author>Dixon, J.K.</author></authors></contributors><titles><title>Purification	6.8×10^{-4} M or 0.03 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle

				and surface tension properties of alkyl sodium sulfosuccinates</title><secondary-title>Journal of Colloid Science</secondary-title></titles><periodical><full-title>Journal of Colloid Science</full-title></periodical><pages>452-459</pages><volume>12</volume><number>5</number><dates><year>1957</year></dates><urls></urls></record></Cite></EndNote>]	concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]

Cationic Surfactants					
Chemical Name in Text	Other Relevant Names	Criteria 1		Criteria 2	Criteria 3
		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
benzalkonium chloride (BAC) CASRN: 8001-54-5	CAS Name: quaternary ammonium compounds, alkylbenzyldimethyl, chlorides	alkyl chains are C12, C14, C16 and C18 and benzyl group	quaternary nitrogen	37 mN/m at concentrations greater than about 4×10^{-4}M and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Nandni</Author><Year>2013</Year><RecNum>14766</RecNum><DisplayText>[47]</DisplayText><record><rec-number>14766</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027033">14766</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nandni,	C12: reported values range from 2.3 - 8.5×10^{-3} M or 0.078 - 0.29 wt% at 25°C C14: 3.7×10^{-4} M or 0.014 wt% and ~25°C (temperature not stated; assumed to be room temperature) C16: 4.2×10^{-5} M or 0.0016 wt% at 23°C C18: reported values range from 7.1 - 8.5×10^{-6} M or 0.0003 - 0.00036 wt% at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[41]</Display

[PAGE]

				D.</author><author>Mahajan, R.K.</author></authors></contributors><titles><title>Micellar and Interfacial Behavior of Cationic Benzalkonium Chloride and Nonionic Polyoxyethylene Alkyl Ether Based Mixed Surfactant Systems</title><secondary-title>Journal of Surfactants and Detergents</secondary-title></titles><periodical><full-title>Journal of Surfactants and Detergents</full-title></periodical><pages>587-599, https://doi.org/10.1007/s11743-012-1427-z</pages><volume>16</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]	Text><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC

					20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
didecyldimethyl ammonium chloride (DDAC) CASRN: 7173-51-5	CAS Name: 1-decanaminium, N-decyl-N,N-dimethyl-, chloride (1:1)	decyl groups	quaternary nitrogen	25.82 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[48]</DisplayText><record><rec-number>14771</rec-number><foreign-	0.39 g/L or 0.039 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[48]</DisplayText><record><rec-number>14771</rec-number><foreign-

				keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027946">14771</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-	keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027946">14771</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-

				dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	dossier/-/registered-dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]

*Not all of the surface tension measurement references identified are run at exactly 20°C, but they are sufficiently close (within 5°C) so as not to affect the measurement. In addition, several measurements were run at 0.1% instead of the recommended 0.5%. Increasing the concentration to 0.5% is likely to lower the surface tension.

**Carboxylic acid compounds, such as oleoyl sarcosine, have a carboxyl group pKa value of ~5, thus at physiological pH values maintained near 7 in the lung, the carboxyl group will be 99% in the anionic form according the Henderson-Hasselbalch equation. Since sodium is the major cation in mammalian body fluids (~145 mM), the use of the sodium oleoyl sarcosine surface tension value is appropriate for its characterization.

***Amphoteric: At pH 7, 90% expected to be nonionic; only small amount cationic.

Hazard Identification

There is concern for dysfunction of mucus, epithelial lining fluid, and natural surfactant lining in the various regions of the respiratory tract from inhalation of surfactants. There is also evidence that some surfactants or similar structures may also interfere with the cell membrane of the epithelium in these same regions [ADDIN EN.CITE ADDIN EN.CITE.DATA]. This effect on cell membranes is apparent from data on numerous surfactants indicating irritation to the skin and eye, as noted below. The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in both human volunteers and in laboratory animals [51, 5-7]. The respiratory tract responses to inhaled surfactant aerosol is thought to be in proportion to the exposure concentration and duration, but available data on acute and repeated-dose effect levels are limited within each subcategory, which limits establishing a correlation between chemical properties and toxicity due to exposure methods (*e.g.,* generated aerosol droplet size).

Nonionic Surfactants

In vivo studies

Several studies were identified for the nonionic siliconized superinone respiratory detergent, 4-(1,1,3,3-tetramethylbutyl)phenol polymer with formaldehyde and oxirane (CASRN 25301-02-4; commonly known as Defomarie, Alevaire, and Tyloxapol). Healthy human volunteers demonstrated significantly decreased respiratory compliance following acute inhalation of Defomaire [ADDIN EN.CITE

<EndNote><Cite><Author>Obenour</Author><Year>1963</Year><RecNum>13656</RecNu

m><DisplayText>[51]</DisplayText><record><rec-number>13656</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1479320595">13656</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Obenour, R. A.</author><author>Saltzman, H. A.</author><author>Sieker, H. O.</author><author>Green, J. L.</author></authors></contributors><titles><title>Effects of surface-active aerosols and pulmonary congestion on lung compliance and resistance</title><secondary-title>Circulation</secondary-title><alt-title>Circulation</alt-title></titles><periodical><full-title>Circulation</full-title><abbr-1>Circulation</abbr-1></periodical><alt-periodical><full-title>Circulation</full-title><abbr-1>Circulation</abbr-1></alt-periodical><pages>888-92</pages><volume>28</volume><edition>OBENOUR, R A
SALTZMAN, H A
SIEKER, H O
GREEN, J L
1963/11/01</edition><keywords><keyword>Aerosols</keyword><keyword>Alcohols</keyword><keyword>Ethanol</keyword><keyword>Heart Failure</keyword><keyword>Humans</keyword><keyword>Infusions, Parenteral</keyword><keyword>Injections, Intravenous</keyword><keyword>Lung</keyword><keyword>Lung Compliance</keyword><keyword>Pulmonary Edema</keyword><keyword>Respiratory Function Tests</keyword><keyword>Silicones</keyword><keyword>Sodium Chloride</keyword><keyword>Surface-Active Agents</keyword></keywords><dates><year>1963</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0009-7322 (Print)
0009-7322 (Linking)</isbn><accession-num>14079193</accession-num><call-num>0 (Aerosols)
0

(Alcohols)
0 (Silicones)
0 (Surface-Active Agents)
3K9958V90M (Ethanol)
451W47IQ8X (Sodium Chloride)</call-num><urls></urls><remote-database-provider>NLM</remote-database-provider><language>Eng</language></record></Cite></EndNote>]. An increased minimum surface tension due to detergent was shown to be dose-dependent, using pulmonary surfactant extracted from dogs with the nonionic surfactant tyloxapol (Alevaire) *in vitro* [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, *in vivo* exposure of dogs to Alevaire (8-hour aerosol exposure; vehicle, particle size and distribution, and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension). The results did not support the dose-dependence of the effect and indicated that small amounts of detergent in the lungs may not detectably alter the surface tension-surface area relationship and that alteration of surface tension is unlikely to occur during reasonable use although there is considerable uncertainty regarding the internal dose achieved [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Inhalation studies using dogs and/or sheep exposed to nonionic surfactant, tyloxapol, resulted in reduced oxygen content of arterial blood due to impaired gas exchange in the lung, increased pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In the study by Modell *et al.* (1969) [ADDIN EN.CITE ADDIN EN.CITE.DATA], no gross pathology differences were seen in detergent-exposed versus control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid

aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl) since these effects were not observed in lungs treated with a less dense aerosol. Normal appearances were observed in the remaining areas of the lungs.

In rodents, irritation and inflammatory effects in the entire respiratory tract have been observed with varying degrees of severity. Acute inhalation exposure *via* nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/L (5,100 mg/m^3) with an MMAD of 2.2 µm and a GSD of 2 to Sorbitan monolaurate, ethoxylated (CASRN 9005-64-5), a chemical not irritating to the skin or eyes [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14776</RecNum><DisplayText>[52]</DisplayText><record><rec-number>14776</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596030693">14776</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated, 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], did not result in an increase in mortalities, clinical signs, or abnormalities in the gross pathology [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14777</RecNum

><DisplayText>[53]</DisplayText><record><rec-number>14777</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596030813">14777</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. A respiratory irritation study using plethysmography was performed on a mixture containing octylphenoxypolyethoxyethanol [ADDIN EN.CITE ADDIN EN.CITE.DATA], which can be severely irritating to the skin and eyes, in male Webster mice exposed for 3 hours to concentrations of 12, 22, 51, 118, and 134 mg/m^3 with 30-60 minutes recovery time (MMAD and GSD not provided). Signs of pulmonary irritation were observed in animals at the two highest concentrations as indicated by a decrease in respiratory frequency (33-58% decrease); this response was preceded by an increase in respiratory frequency (11-12.5% increase) at the highest three concentrations without an increase in gross lung abnormalities, pulmonary edema, or lung weight [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>1992</Year><RecNum>14778</RecNum><DisplayText>[54]</DisplayText><record><rec-number>14778</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035219">14778</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Alarie, Y.</author><author>Stock, M.F.</author></authors></contributors><titles><title>Respiratory Irritancy on a Mixture containing Polyethylene Glycol Mono(Octyl)Phenyl Eether CAS #9035-19-5</title><secondary-title>ChemView - U.S. Environmental Protection Agency</secondary-title></titles><periodical><full-title>ChemView - U.S. Environmental Protection Agency</full-title></periodical><pages>37, https://chemview.epa.gov/chemview/proxy?filename=09022526800b76c9_86960000465_09-26-2011_8D_PHCS_Original%20-%2086960000465.pdf</pages><dates><year>1992</year></dates><urls></urls></record></Cite></EndNote>]. An acute inhalation exposure study in Syrian hamsters exposed to 3.0 mg/L of octylphenoxypolyethoxyethanol with varying exposure durations showed that lung deposition directly corresponded to mortality with an LD_{50} of 1300-2100 µg with an MMAD of 1.47 µm and a GSD of 1.84 [ADDIN EN.CITE <EndNote><Cite><Author>Damon</Author><Year>1982</Year><RecNum>13323</RecNum><DisplayText>[55]</DisplayText><record><rec-number>13323</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1479320592">13323</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Damon, E. G.</author><author>Halliwell, W. H.</author><author>Henderson, T. R.</author><author>Mokler, B. V.</author><author>Jones, R. K.</author></authors></contributors><titles><title>Acute toxicity of polyethylene glycol p-isooctylphenol ether in syrian hamsters exposed by inhalation or bronchopulmonary lavage</title><secondary-title>Toxicology and applied pharmacology</secondary-title><alt-

title>Toxicol Appl Pharmacol</alt-title></titles><periodical><full-title>Toxicology and Applied Pharmacology</full-title><abbr-1>Toxicol. Appl. Pharmacol.</abbr-1></periodical><pages>53-61</pages><volume>63</volume><number>1</number><edition>Damon, E G
Halliwell, W H
Henderson, T R
Mokler, B V
Jones, R K
1982/03/30</edition><keywords><keyword>Animals</keyword><keyword>Cricetinae</keyword><keyword>Detergents/ toxicity</keyword><keyword>Dose-Response Relationship, Drug</keyword><keyword>Female</keyword><keyword>Lethal Dose 50</keyword><keyword>Lung/ drug effects/pathology</keyword><keyword>Male</keyword><keyword>Mesocricetus</keyword><keyword>Octoxynol</keyword><keyword>Polyethylene Glycols/administration & dosage/ toxicity</keyword><keyword>Surface-Active Agents/ toxicity</keyword><keyword>Therapeutic Irrigation</keyword></keywords><dates><year>1982</year><pub-dates><date>Mar 30</date></pub-dates></dates><isbn>0041-008X (Print)
0041-008X (Linking)</isbn><accession-num>7071873</accession-num><call-num>0 (Detergents)
0 (Surface-Active Agents)
30IQX730WE (Polyethylene Glycols)
9002-93-1 (Octoxynol)</call-num><urls></urls><remote-database-provider>NLM</remote-database-provider><language>Eng</language></record></Cite></EndNote>]. The deaths in these animals were attributed to severe laryngeal edema and ulcerative laryngitis while the lower airways in these animals were relatively free of serious pathologies which likely indicates limited deposition to the lower airways in this study. The authors hypothesized that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the chemical resulted in a large concentration on the laryngeal mucosa,

though laryngeal deposition is typically a function of aerodynamics. In the only 2-week whole-body inhalation study for nonionic surfactants, male and female Sprague-Dawley rats were exposed to 5.3 and 10.3 mg/m^3 (5/sex/dose; MMAD 1.8 µm, GSD 1.8) octylphenoxypolyethoxyethanol for 6 hours/day, 5 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge; a LOAEC of 5.3 mg/m^3 was identified.

Mechanistic studies

In vitro studies of surfactant on cell membranes have provided evidence of possible modes of action (MOAs). Warisnoicharoen *et al.* (2003) [ADDIN EN.CITE ADDIN EN.CITE.DATA] evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$; CASRN 9004-98-2), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$; CASRN 9002-92-0), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) on submerged cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay by exposing the cells to 0.1mL of the serially diluted microemulsion (particle size not reported) for 30 minutes followed by a 60 minute incubation with a MTT solution. All surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg *et al.* (2019) [ADDIN EN.CITE <EndNote><Cite><Author>Lindenberg</Author><Year>2019</Year><RecNum>14779</RecNum><DisplayText>[57]</DisplayText><record><rec-number>14779</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035601">14779</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lindenberg, F.</author><author>Sichel, F.</author><author>Lechevrel, M.</author><author>Respaud, R.</author><author>Saint-Lorant, G.</author></authors></contributors><titles><title>Evaluation of Lung Cell Toxicity of Surfactants for Inhalation Route</title><secondary-title>Journal of Toxicology and risk assessment</secondary-title></titles><periodical><full-title>Journal of Toxicology and risk assessment</full-title></periodical><pages>https://doi.org/10.23937/2572-4061.1510022</pages><volume>5</volume><number>1</number><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] evaluated the cytotoxic activity of the three nonionic polymeric surfactants Polysorbate 20 (CASRN 9005-64-5), Polysorbate 80 (Tween 80) and Poloxamer 188 (CASRN 691397-13-4), which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, in a BEAS-2B human bronchial epithelial cell model using an innovative air-liquid interface (ALI) method of exposure with a nasal spray system (MMAD and GSD not provided). In this study, the ALI results were compared to the classical submerged cell culture or liquid/liquid (L/L) model. The study measured the release of lactate dehydrogenase (LDH), an intercellular enzyme present in the cytoplasm, indicative of the loss of membrane integrity. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method;

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however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to a lesser extent, Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any *in vitro* cytotoxicity.

The available *in vitro* and *in vivo* data indicate inconsistency in respiratory toxicity among nonionic surfactants; however, the degree to which the variation is due to experimental design or bioactivity of the surfactant is not discernible from these data. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties, such as surface tension or CMC. Similarly, the examination of the relationship between chemical properties of nonionic surfactants and eye irritation has not established that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths can be used to predict eye irritation potential across the nonionic surfactant subcategory [ADDIN EN.CITE <EndNote><Cite><Author>Heinze</Author><Year>1999</Year><RecNum>14780</RecNum><DisplayText>[58]</DisplayText><record><rec-number>14780</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035990">14780</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Heinze, J.E.</author><author>Casterton, P.L.</author><author>Atrash, J.</author></authors></contributors><titles><title>Relative Eye Irritation Potential of Nonionic Surfactants: Correlation to Dynamic Surface Tension</title><secondary-title>Journal of toxicology: cutaneous and ocular toxicology</secondary-title></titles><periodical><full-title>Journal of toxicology: cutaneous and ocular toxicology</full-

title></periodical><pages>359-374,
https://doi.org/10.3109/15569529909065552</pages><volume>18</volume><dates><year>199
9</year></dates><urls></urls></record></Cite></EndNote>]. However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or higher surfactant concentration when surface tension was measured under dynamic conditions (0.24, 1, and 4 bubbles/second). Whether this chemical property similarly predicts potency of nonionic surfactants for respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

In vivo studies

Two acute inhalation toxicity studies were identified for anionic surfactants, both demonstrated high toxicity *via* the inhalation route. Oleoyl sarcosine (CASRN 110-25-8), irritating to the skin and damaging to the eye [ADDIN EN.CITE
<EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14781</RecNum
><DisplayText>[59]</DisplayText><record><rec-number>14781</rec-number><foreign-
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Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-
(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Skin
irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-

title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/4/2/?documentUUID=fbaef057-ecc7-4763-aa56-1fa2c88c606c</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], was evaluated in a 4-hour nose-only inhalation study in male and female Sprague-Dawley rats at concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L (300, 600, 2,200, 3,700 mg/m^3). The MMAD and GSD were not reported. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For sodium lauroyl sarcosinate (CASRN 137-16-6), irritating to the skin and corrosive to the eye (undiluted) [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum><DisplayText>[60]</DisplayText><record><rec-number>14782</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036284">14782</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], 5 male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L (50, 500, 1,000, and 5,000 mg/m^3) with a MMAD of 4.4, 2.9, 3.7, and 6.0 µm; and GSD of 2.7, 3, 4.2, and 2.9, respectively. Additionally, 5 female rats were exposed

to 1.1 or 5.5 mg/L with a MMAD 3.7 or 6.0 µm and GSD of 4.2 or 2.9, respectively [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum><DisplayText>[60, 61]</DisplayText><record><rec-number>14782</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036284">14782</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14783</RecNum><record><rec-number>14783</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036540">14783</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-

dossier/14123/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. The 5 mg/L dose resulted in fatality in all 10 animals (males and females) tested within 1-2 h of dosing and the 0.5 mg/L dose resulted in fatality for 4/5 of the males and exposure to 1 mg/L resulted in fatalities for the 10 animals (males and females) within 1-2 days of exposure. Males exposed to 0.05 mg/L did not demonstrate any adverse clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in males and females receiving concentrations of ≥ 0.5 mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Repeated-dose inhalation studies were identified for oleoyl sarcosine, and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (6 hours/day, 5 days/week; Organization for Economic Cooperation and Development [OECD] Test Guideline [TG] 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L (0, 6, 20, or 60 mg/m^3). The particle exposure MMAD was 1.11, 1.15, or 1.22 μm, GSD 1.68-2.57, and density 0.79 g/cm^2 for 6 hours/day, 5 days/week in 10% ethanol [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum><DisplayText>[62]</DisplayText><record><rec-number>14784</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-

title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes were observed in male animals at the high exposure concentration. In female animals of the mid-concentration exposure group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals at the mid and high concentrations, which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the alveoli walls and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The LOAEC was 0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

Dioctyl sulfosuccinate sodium salt (DOSS; CASRN 577-11-7) was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex). Rats were exposed to an aerosol of a product containing 0.0042 mg/L (4.2 mg/m^3) DOSS, for 4 hours a day, 5 days a week (as reported in a secondary source; exposure details, MMAD, and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>CIR</Author><Year>2013</Year><RecNum>14785</RecNum><DisplayText>[63]</DisplayText><record><rec-number>14785</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

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timestamp="1596037107">14785</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>CIR</author></authors></contributors><titles><title>Safety Assessment of Alkyl Sulfosuccinate Salts as Used in Cosmetics, Re-Review, CIR Expert Panel Meeting, June 10-11, 2013</title><secondary-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</secondary-title></titles><periodical><full-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</full-title></periodical><pages>171, https://www.cir-safety.org/sites/default/files/Sulfosuccinates_RR.pdf</pages><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]. There were no statistically significant differences in exposed and control groups for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood as indicated by elevated erythrocytic values (not otherwise specified) at 7 weeks and depressed mean corpuscular hemoglobin concentration values at 13 weeks in male rats. In females, depressed serum glutamic pyruvic transaminase and significant effect on absolute heart weight was observed at 7 weeks, depressed serum alkaline phosphatase was observed at 13 weeks and elevated glucose at 7 and 13-weeks. At 7 weeks, the lungs of necropsied animals showed scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single exposed male rat. A LOAEC of 4.2 mg/m^3 was identified based on the blood effects in male rats.

Mechanistic studies

Mechanistic studies on the pulmonary effects of anionic surfactants have been studied in dogs, rabbits, and sheep exposed to DOSS.

Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to DOSS in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Anesthetized dogs were exposed *via* a ventilator to particle sizes of 0.5 to 15 μm with an MMAD of 3 μm (no GSD reported). Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Alveolar-capillary barrier permeability studies using radiolabeled aerosol tracers have evaluated whether detergents effect the surfactant layer to increase alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary elimination of radiolabeled diethylenetriamine pentaacetic acid (DTPA; CASRN 67-43-6) a relatively small hydrophilic molecule, indicating an increased alveolar permeability after detergent exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In most studies, this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occurs with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in rabbits exposed to multiple dilutions (0.125, 0.25, 0.5, and 2%) with a MMAD of 1.7 μm of the liquid detergent [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Studies also evaluated the elimination of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser

degree than DTPA [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Wang *et al.* (1993) [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which was attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies was hypothesized to be a result of increased alveolar surface tension, which may result in increased permeability by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, as noted, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Cationic Surfactants

In vivo studies

Three acute inhalation toxicity studies were identified for cationic surfactants; one study each for DDAC, dioctadecyldimethylammonium chloride (DODMAC; CASRN 107-64-2), and BAC. DDAC, which is corrosive to the skin and severely damaging to the eye [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14786</RecNum><DisplayText>[71]</DisplayText><record><rec-number>14786</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038295">14786</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/5864/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], was tested in rats (5/sex/dose, unspecified strain) exposed *via* inhalation to 0.05, 0.09, 0.13, 0.25, 1.36, or 4.54 mg/L (50, 90, 130, 250, 1,360, or 4,540 mg/m^3) for 2 hours with an observation period of 14 days (no additional exposure conditions reported). An LC_{50} of 0.07 mg/L was identified based on unspecified abnormalities identified in several organs including the lungs [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2006</Year><RecNum>14845</RecNum><DisplayText>[72]</DisplayText><record><rec-number>14845</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1597755265">14845</key></foreign-keys><ref-type name="Journal

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Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Reregistration Eligibility Decision for Aliphatic Alkyl Quanternaries (DDAC)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>127, https://archive.epa.gov/pesticides/reregistration/web/pdf/ddac_red.pdf</pages><volume>EPA739-R-06-008</volume><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]. A similar quaternary amine, DODMAC, which is irritating to the skin and causes serious damage to the eyes [ADDIN EN.CITE <EndNote><Cite><Author>EURAR</Author><Year>2009</Year><RecNum>14787</RecNum><DisplayText>[73]</DisplayText><record><rec-number>14787</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038841">14787</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EURAR</author></authors></contributors><titles><title>European Union Risk Assessment Report (EURAR), CAS No: 107-64-2, EINECS No: 203-508-2, dimethyldioctadecylammonium chloride (DODMAC)</title><secondary-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau

(ECB)</secondary-title></titles><periodical><full-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</full-title></periodical><pages>123, https://echa.europa.eu/documents/10162/46f2f114-12ff-4af4-8da7-72148b6a202e</pages><volume>14</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], was tested in albino rats (10 males, strain not specified) to the test substance (1:29 distilled water) *via* inhalation at 180 mg/L (180,000 mg/m^3) for one hour and observed for 14 days (no additional exposure conditions reported). No mortalities were reported and observed treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose, and labored respiration. All animals appeared normal one day after dosing. The LC_{50} (1 h) was > 180 mg/L. BAC, which is corrosive to the skin and causes severe eye damage [ADDIN EN.CITE ADDIN EN.CITE.DATA], was tested in female Wistar rats (5/group) exposed *via* nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (MMAD and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>Swiercz</Author><Year>2008</Year><RecNum>14789</RecNum><DisplayText>[75]</DisplayText><record><rec-number>14789</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039305">14789</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Swiercz, R.</author><author>Halatek, T.</author><author>Wasowicz, W.</author><author>Kur, B.</author><author>Grzelińska, Z.</author><author>Majcherek, W.</author></authors></contributors><auth-

address>Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Łódź, Poland. radek@imp.lodz.pl</auth-address><titles><title>Pulmonary irritation after inhalation exposure to benzalkonium chloride in rats</title><secondary-title>Int J Occup Med Environ Health</secondary-title><alt-title>International journal of occupational medicine and environmental health</alt-title></titles><periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></periodical><alt-periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></alt-periodical><pages>157-63</pages><volume>21</volume><number>2</number><edition>2008/08/22</edition><keywords><keyword>Animals</keyword><keyword>Benzalkonium Compounds/administration & dosage/*toxicity</keyword><keyword>Female</keyword><keyword>Inhalation Exposure</keyword><keyword>Lung Diseases/*chemically induced/pathology</keyword><keyword>Organ Size/drug effects</keyword><keyword>Rats</keyword><keyword>Rats, Wistar</keyword></keywords><dates><year>2008</year></dates><isbn>1232-1087 (Print)
1232-1087</isbn><accession-num>18715840</accession-num><urls></urls><electronic-resource-num>10.2478/v10001-008-0020-1</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The LC_{50} was reported to be approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as tumor necrosis factor (TNF)-a, interleukin (IL)-6. Indicators of respiratory tract damage, including increased LDH, total protein, and lung weight were also observed.

Three repeated dose inhalation studies of three different exposure durations were identified for DDAC: 14-day, 28-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed *via* whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Lim</Author><Year>2014</Year><RecNum>14790</RecNum><DisplayText>[76]</DisplayText><record><rec-number>14790</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039544">14790</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lim, C. H.</author><author>Chung, Y. H.</author></authors></contributors><auth-address>Toxicity Research Team, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of didecyldimethylammonium chloride on sprague-dawley rats after two weeks of inhalation exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>205-10</pages><volume>30</volume><number>3</number><edition>2014/10/25</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword></keywords><dates><year>2014</year><

pub-dates><date>Sep</date></pub-dates></dates><isbn>1976-8257 (Print)
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In the intermediate exposure (4-week) study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed *via* dynamic nose-only inhalation to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 DDAC (MMAD 1.4, 1.5, and 1.9 µm, GSD 1.83, 1.86, and 1.87, density not reported) for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington,

D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] . Body weights were significantly reduced in the high exposure group (males only) on days 14, 21, and 25. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. BALF analysis indicated that, at the high concentration, neutrophils and eosinophils increased with a concomitant decrease in macrophages. Histopathological findings in the nasal cavity were graded according to severity from minimal to severe and increased mucus of the respiratory epithelium in males and females was minimal to moderate at all exposures and mild to moderate ulceration of the nasal cavity in males and females in the high concentration group only. In males, there was an increase in cell count and total protein across all exposures. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. A conservative LOAEC of 0.08 mg/m^3 was previously identified by the Agency based on increased mucus of the respiratory epithelium and increased LDH; however, due to the mild effects and low number of animals/group, the effects were not statistically significant [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-

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In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole-body exposure chambers for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2017</Year><RecNum>14736</RecNum><DisplayText>[77]</DisplayText><record><rec-number>14736</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018905">14736</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, Y. S.</author><author>Lee, S. B.</author><author>Lim, C. H.</author></authors></contributors><auth-address>Chronic Inhalation Toxicity Research Center, Chemicals Toxicity Research Bureau, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of Didecyldimethylammonium Chloride (DDAC) on Sprague-Dawley Rats after 13 Weeks of Inhalation Exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol

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Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>7-14</pages><volume>33</volume><number>1</number><edition>2017/01/31</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword><keyword>Sub-chronic</keyword></keywords><dates><year>2017</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>28133508</accession-num><urls></urls><custom2>PMC5266374</custom2><electronic-resource-num>10.5487/tr.2017.33.1.007</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The MMAD of the DDAC aerosol was 0.63 μm, 0.81 μm, and 1.65 μm, and the geometric standard deviations were 1.62, 1.65, and 1.65 in the low (0.11 ± 0.06 mg/m^3), the middle (0.36 ± 0.20 mg/m^3) and the high (1.41 ± 0.71 mg/m^3) exposure groups, respectively. Body weight influenced by exposure to DDAC with the mean body weight approximately 35% lower in the high exposure (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high exposure (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and LDH were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups and in males in the high concentration group only, while inflammatory cell infiltration and interstitial pneumonia was observed in both the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

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BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations of 0.8, 4 and 20 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Mean concentration of BAC in the whole-body exposure chambers of the T1 (0.8 mg/m^3), T2 (4 mg/m^3) and T3 (20 mg/m^3) groups during the exposure period was 0.84 ± 0.09, 4.01 ± 0.12, and 19.57 ± 0.97 mg/m^3, respectively; the MMAD of the aerosols was 1.614, 1.090, and 1.215 μm, respectively, and the GSD was 2.00, 1.86, and 1.51, respectively. The MMAD and GSD were confirmed to be within the range recommended by the OECD (2018) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Among the general signs observed

during the exposure period, soiled perineal region, rales, and discharge were continuously observed during the 2-week recovery period. Rales and deep respiration were observed in the high concentration. Exposure-related effects in the upper airway included nasal discharge at the low and mid concentrations, and ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high concentrations.

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In the lower airways, degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens were observed in the mid and high concentration male groups and high concentration dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchioles were observed in both males and females. Effects indicating tissue injury included squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole. In the BALF analysis, the concentration of reactive oxygen species (ROS)/reactive nitrogen species (RNS), IL-1β, IL-6, and macrophage inflammatory protein (MIP)-2 decreased concentration-dependently at the end of the exposure period, which indicated oxidative damage, but did not show a concentration-dependent change at 4 weeks of recovery. The concentrations of TNF-α, IL-4, and transforming growth factor (TGF)-β did not show changes associated with test substance exposure. Relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors identified a LOAEC of 0.8 mg/m^3 based on effects in the nasal cavity.

Mechanistic studies

In vitro assays have demonstrated that cytotoxic effects of cationic surfactants have significantly greater toxicity to non-polarized than polarized mammalian cells [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In this study, cell viability as measured by LDH and MTT assays in non-polarized HeLa immortal cell line cells and fetal skin dendritic cells (FSDC) was more sensitive to the effects of different cationic surfactants of varying alkyl chain length and polar head groups than polarized cell lines Madin-Darby Canine Kidney (MDCK) and Caco-2. The cationic surfactant toxicity was shown to occur well below their CMC, and greater toxicity was observed with alkyl lengths of 10-12 than 14-16; however, this association was not strictly a linear relationship. In addition, the cationic surfactants with a larger polar head group (*i.e.*, benzalkonium) were 2-5 times more toxic than cationic surfactants with a more localized charge (*i.e.*, trimethylammonium).

The effects of BAC on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells has been replicated *in vitro* using a dynamic culture condition that reflects the natural microenvironment of the lung to simulate the contraction and expansion of breathing [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Normal breathing levels were simulated (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of exposure rate during the cell culture. The system assessed toxicity by culturing submerged cells with different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following a 24-hr exposure to BAC, cellular metabolic activity, IL-8, and ROS levels were

significantly affected, compared to untreated cells, when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed a higher toxic response to BAC as indicated by increased ROS levels.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The animal inhalation toxicity data identified by the literature search and PODs from the studies are summarized in [REF _Ref46931035 \h * MERGEFORMAT]. It should be emphasized that new information (*e.g.*, study data, POD derivation approaches, mechanistic information, *etc.*) may lead to updates/additions to this table. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human equivalent inhalation exposure [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-

11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. The exposure duration adjustment and DAF approaches were described above. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in [REF _Ref46931035 \h * MERGEFORMAT] for each of the toxicity studies from which PODs could be identified. However, other approaches to dosimetry adjustment may be considered relevant (*e.g.*, use of the multiple-path particle dosimetry model [MPPD]).

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For the nonionic surfactant, octylphenoxypolyethoxyethanol, the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with effects in the thoracic region; therefore, the RDDR of 0.812 was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, the extrathoracic RDDR (0.0.111) was used to calculate the HEC. In the 28-day inhalation study with DDAC, effects were observed throughout the respiratory tract, including the nasal cavity; therefore, the thoracic RDDR (0.854) was used for calculating the HEC. Similarly, for the cationic surfactant, BAC histopathological cellular changes were observed in the nasal cavity and lungs, indicating the extrathoracic RDDR (0.106) should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in [REF _Ref46931035 \h * MERGEFORMAT].

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Table [SEQ Table * ARABIC]. Inhalation Toxicity Points of Departure and Human Equivalent Concentrations (HEC) for Surfactants.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/Type	Study POD	Value (mg/m^3)	Reference	Density (g/cm^3) at 20 °C[1]	RDDR Model Input Parameters		RDDR[2]	HEC (mg/m^3)
							MMAD (μm)	GSD		
Nonionic	octylphenoxypolyethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body	LOAEC	5.3	[ADDIN EN.CITE <EndNote><Cite><Author>MDEQ</Author><Year>2003</Year><RecNum>14731</RecNum><DisplayText>[8]</DisplayText><record><rec-number	0.998 water vehicle	1.80	1.80	$RDDR_{ET}$ = 0.196 $RDDR_{TB}$ = 1.367 $RDDR_{PU}$ = 0.564 **$RDDR_{TH}$ = 0.812** $RDDR_{TOT}$ = 1.547	1.0 7.2 3.0 **4.4** 8.2

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					><auth or>MD EQ</au thor></ authors ></cont ributors ><titles ><title >To: Memo to File for Triton X-100 (CAS # 9002- 93-1); From: Gary Butterfi eld; Date: Novem ber 21,200 3; Subject : Screeni ng level for Triton					

					X-100 (CAS # 9002-93-1)</title><secondary-title>Michigan Department of Environmental Quality (MDEQ)</secondary-title></titles><periodical><full-title>Michigan Department of Environmental Quality (MDEQ)</full-title></					

					periodical><pages>2</pages><dates><year>2003</year></dates><urls></urls></record></Cite></EndNote>]					
Anionic	oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD TG 412)	LOAEC	< 6	[ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum><Di	0.7893 ethanol vehicle	1.16	2.12	**$RDDR_{ET}$ = 0.111** $RDDR_{TB}$ = 2.008 $RDDR_{PU}$ = 0.447 $RDDR_{TH}$ = 0.742 $RDDR_{TOT}$ = 0.970	**< 0.6** < 12.0 < 2.7 < 4.5 < 5.8

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					d dose toxicity : Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registr					

					ation-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]					
Cationic	DDAC	4-week, 6 hr/d, 5 d/wk; nose-only	LOAEC[3] (lung effects)	0.08	[ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year>	NR	1.60	1.85	$RDDR_{ET}$ = 0.211 $RDDR_{TB}$ = 1.674 $RDDR_{PU}$ = 0.539 **$RDDR_{TH}$ = 0.854** $RDDR_{TOT}$ = 1.607	0.02 0.13 0.04 **0.07** 0.13

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					Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016<					

					/year> </dates> <urls> </urls></record> </Cite> </EndNote>]					
	BAC	14-day, 6 hr/d, 7 d/wk; whole body	LOAEC (nasal effects)	0.8	[ADDIN EN.CITE ADDIN EN.CITE.DATA]	0.998 water vehicle 2% dose solution	1.31	1.79	**$RDDR_{ET}$ = 0.106** $RDDR_{TB}$ = 1.988 $RDDR_{PU}$ = 0.528 $RDDR_{TH}$ = 0.815 $RDDR_{TOT}$ = 0.991	**0.08** 1.59 0.42 0.65 0.79

MMAD: Mass Median Aerodynamic Diameter of inhalation study aerosol, average values listed; GSD: Geometric Standard Deviation of the inhalation study aerosol, average values listed; RDDR: Regional Deposited Dose Ration; ET: Extrathoracic; TB: Tracheobronchial; PU: Pulmonary; TH: Thoracic = TB + PU; TOT = ET + TB + PU.

[1]Exact density of administered compounds not reported (NR); vehicle density was listed when provided.

[2]RDDR values are for male and female animals, whichever was lower, as calculated using RDDR.exe and described in the Supporting Information file at "Section 2 RDDR Modeling".

[3]conservative estimate: effects were not statistically significant.

NA: Data not available or RDDR values could not be calculated from the available information.

Benchmark Margin of Exposure Analysis

The substances shown in [REF _Ref46931035 \h * MERGEFORMAT] provide representative examples of PODs that may be applied to new chemistries that meet the Surfactant Criteria, after evaluating whether the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] are appropriate toxicological analogues for read-across to the new chemical substance. Alternatively, the notifier may propose a different representative POD and/or analogue, if supported by scientific evidence. If a determination cannot be made on whether one of these chemical substances ([REF _Ref46931035 \h * MERGEFORMAT] or other representative analogue) is an appropriate toxicological analogue, then the relevant substance from [REF _Ref46931035 \h * MERGEFORMAT] should be identified as a comparator substance[4] for use in the Tiered-Testing Strategy, discussed below. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs or consideration of the representativeness and comprehensiveness of the available database to characterize potential effects after inhalation exposure. As shown in [REF _Ref46931035 \h * MERGEFORMAT], the uncertainty factors were based on RDDRs that were used as DAFs to account for animal-to-human toxicokinetic differences.

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[4] A comparator substance is one that may possess similar properties to the new chemical substance and for which inhalation toxicity data are available. EPA may "read-across" the toxicity data from the comparator substance to the new chemical substance when no other information is available. The tiered-testing approach for this category is designed to determine whether this practice may be refined or supported by additional data. As such, the comparator substance should be used in side-by-side testing in Tiers I-III with a new chemical substance to aid with interpreting the test results of the new chemical substance.

In the case of surface-active substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (See, *e.g.*, EPA, 2020 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14794</RecNum><DisplayText>[82]</DisplayText><record><rec-number>14794</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040494">14794</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>84, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2013-0605-0051&contentType=pdf</pages><volume>EPA-HQ-OPP-2013-0605-0051</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a direct-acting ~~adverse outcome pathway (AOP)~~ mode of action (MOA) such as the one

regarding surfactant that is under development [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], the default values for UF_H and UF_A are each reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1. In order to apply these reductions, the following criteria must be established:

1. A description of the MOA~~AOP~~,
2. A discussion of why the MOA~~AOP~~ is unlikely to differ between humans, in the case of UF_H, or between animals in comparison to humans, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is addressed by the use of dosimetry modeling.

When the above criteria are met, application of the appropriate DAF (*e.g.*, the RDDR for particles) should still be applied, given that deposition is the most appropriate dose metric for

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assessing acute/subacute effects from surface-active agents. However, since the DAF accounts for the toxicokinetic component of UF_A, the remaining value of 3 (*i.e.*, $10^{0.5}$ or 3.16) should be retained for the toxicodynamics component of the UF_A.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all three of the above criteria, then a value of 3 may be applied, which reflects ~~. The reduced value represents~~ a reduction in the TK component ~~of this UF~~ to 1 and application of a value of 3 for the ~~, with the remaining value of 3 accounting for the~~ TD component.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of dosimetric adjustments for ~~quantifying~~ deriving a ~~an~~ HEC or ~~when the available information~~ does not support each of the above three criteria. If the available information allows derivation of a ~~an~~ HEC and/or application of the above criteria, then a value of 3 may be applied, which reflects ~~presents~~ a reduction in the TK component to 1 and application of a value of 3 for the TD component.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL with an

appropriate biologically significant benchmark response (*e.g.*, 10% extra risk for quantal data or 1 standard deviation for continuous data) should be calculated and a value of 1 should be assigned to this ~~applied for this area of~~ uncertainty factor.

The above considerations and approaches support the application of a benchmark MOE ranging from 10 (*i.e.*, $10^{0.5} \times 10^{0.5} \approx 10$) to 1,000 depending on the chemical substance identified as an appropriate toxicological analogue and available data on the new chemical substance. In those instances where the data are too limited to determine whether ~~n~~ one of the chemical substances in Table 3 is appropriate for extrapolating ~~the hazards~~ to the new chemical substance, experimental testing should be performed to ~~aid with~~ informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

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The assessment framework outlined includes a number of uncertainties and limitations, including those associated with extrapolating the hazards identified from the chemical substances shown in [REF _Ref46931035 \h * MERGEFORMAT]. Uncertainties associated with using animals to estimate human toxicity are recognized and methods are presented to reduce extrapolation uncertainties [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2014</Year><RecNum>14795</RecNum><DisplayText>[84]</DisplayText><record><rec-number>14795</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040729">14795</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance on Grouping of Chemicals, Second Edition, Series on Testing & Assessment</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>141, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en</pages><volume>ENV/JN/MONO(2014)4</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. Procedures for the adjustment of exposure durations for inhalation exposures and application of DAFs to derive HECs are well-established procedures for reducing uncertainties associated with the TK aspects of animal-to-human extrapolation ~~factors~~ and derivation of benchmark MOEs ~~(*i.e.*, type and magnitude of uncertainty factors)~~ [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 20]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

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20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Likewise, EPA ~~has~~ recommends ~~ed~~ that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of the chemical substances in [

REF _Ref46931035 \h * MERGEFORMAT] to new chemical substances needs to be carefully considered, with attention given to the influence of additional functional groups on the toxicity of the new chemical substance, as well as the particle properties (MMAD, GSD, and density) of the candidate new chemical substance. Simulation studies using dosimetry models such as the RDDR or multiple-path particle dosimetry (MPPD) models can inform these considerations. Additionally, the risk assessors should consider if a different comparator substance and/or POD may be more appropriate (*e.g.*, based on new scientific information of the new chemical substance profile). Risk assessors should consider the surface tension and CMC criteria ([REF _Ref47613375 \h * MERGEFORMAT]) compared to these measurements for the new chemical substance and the influence of the presence or absence of additional functional groups on these criteria (*e.g.*, would a particular functional group increase or decrease toxicity, for example by another mechanism of action). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, the hazard(s) and risk(s) should be characterized using the chemical substance as a toxicological analogue to the new chemical substance. Of course, uncertainties regarding this extrapolation should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that a chemical substances ([REF _Ref46931035 \h * MERGEFORMAT] or other relevant analogue) is comparable to or represents an acceptable toxicological analogue to the new chemical substance, then the Tiered-Testing Strategy provided could be used to determine whether the new chemical substance has lower, comparable, or higher toxicity to the relevant chemical substance in [REF _Ref46931035 \h * MERGEFORMAT], as a comparator

substance and not as a toxicological analogue. Prior to conducting such testing, the scientific basis for selecting the comparator substance to the new chemical substance should be understood and a rationale provided as to why the comparator substance will be used for testing.

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing

Commented [A31]: Shorten?:
Amy, William, Jane

Reviewer #1 wanted even more explanation of test methods, so not shortening this section much

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]. Moreover, the amended TSCA requires entities undertaking voluntary testing for submission to EPA to first "…attempt to develop the information by means of an alternative test method or strategy …before conducting new vertebrate testing…" [ADDIN EN.CITE

<EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum>
<DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-
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title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53
&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cit
e></EndNote>]. Additionally, in 2019, EPA was directed to prioritize efforts to use NAMs to
reduce animal testing [ADDIN EN.CITE
<EndNote><Cite><Author>Wheeler</Author><Year>2019</Year><RecNum>14797</RecNu
m><DisplayText>[86]</DisplayText><record><rec-number>14797</rec-number><foreign-
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A.R.</author></authors></contributors><titles><title>Directive to Prioritize Effects to Reduce
Animal Testing</title><secondary-title>United States Environmental Protection
Agency</secondary-title></titles><periodical><full-title>United States Environmental
Protection Agency</full-title></periodical><pages>3,
https://www.epa.gov/sites/production/files/2019-09/documents/image2019-09-09-

231249.pdf</pages><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. Multiple NAMs exist which can be used to assess hazards and risks of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing and *in vitro* methods to specifically assess respiratory toxicity. Several methods are described within a tiered-testing strategy recognizing that these assays are provided as examples and the development of NAMs is advancing rapidly. As such, the NAMs included here should not be considered all-inclusive or a final compilation. EPA strongly encourages the development and use of NAMs, particularly to reduce or replace the use of animals and is open to considering and discussing additional NAMs with PMN submitters during a pre-notice consultation [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14829</RecNum><DisplayText>[87]</DisplayText><record><rec-number>14829</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596098792">14829</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Schedule a Pre-Submission Meeting, Reviewing New Chemicals under the Toxic Substances Control Act (TSCA)</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>https://www.epa.gov/reviewing-new-chemicals-under-toxic-

substances-control-act-tsca/forms/program-contacts-and</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

In the interest of reducing or replacing vertebrate testing and designing a scientifically robust testing approach, when a surfactant is determined to be respirable, EPA encourages evaluating its potential to cause respiratory tract toxicity using an AOP approach. The OECD provides "An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect" and that "AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning" [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14798</RecNum><DisplayText>[88]</DisplayText><record><rec-number>14798</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041285">14798</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Adverse Outcome Pathways, Molecular Screening and Toxicogenomics</title><secondary-title>Organization for Economic Cooperation and Development (OECD)</secondary-title></titles><periodical><full-title>Organization for Economic Cooperation and Development (OECD)</full-title></periodical><pages>http://www.oecd.org/env/ehs/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm</pages><dates><year>2020</year></dates><urls></urls></record></Cite>

></EndNote>]. AOPs in various stages of development are useful for different purposes and an AOP may be useful even if it has not been formally evaluated by the OECD.

An AOP can be used to help design a testing strategy and to identify NAMs that can query the key events leading up to the adverse outcome. As an example, using the respiratory contact irritant chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CASRN 1897-45-6), Syngenta Crop Protection applied a NAM for the assessment of inhalation toxicology based on an AOP approach [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The approach involved derivation of the POD from an *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Similar approaches can be used for surfactants where *in vitro/ex vivo* systems may be used to investigate specific key events in an AOP and confirm that a new chemical substance fits within the boundaries of the Surfactant Category, and therefore, may act like a surfactant (group assignment *via* similar AOP) and/or if other substance-specific properties lead to a predominant type of key event within the AOP. Further, *in vitro* tests may deliver information while avoiding *in vivo* testing or, if considered, provide helpful information on dose-selection for *in vivo* testing.

An AOP connects a molecular initiating event (MIE) to key events, at the cellular, tissue, and organ levels, which lead to an adverse outcome at the organism or population level [ADDIN EN.CITE ADDIN EN.CITE.DATA]. For surfactants, proposed MIEs include interaction of the substance with the epithelial lining fluid or lung-surfactant, or the molecular interaction of the substance itself with cell membranes of the epithelium in the respiratory tract. The resulting key events include disruption of airway epithelial cells (AEC) due to loss of lung cell surfactant

function and/or the loss of membrane integrity (cellular level key events). These cellular events may lead to different tissue or organ level events (*e.g.*, cytotoxicity and perturbation of AEC, increased alveolar surface tension and alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (*i.e.*, the adverse outcome) (*e.g.*, pneumonia, limited lung function by chronic obstruction (COPD), interstitial fibrosis, *etc*.).

Commented [A32]: Define CLE here?

Some *in vitro* tests, such as by capillary surfactometer, may be useful in screening chemicals to be tested for the Surfactant Category, but do not by themselves constitute adequate tests for acute respiratory tract effects of these chemicals. This information should be taken into consideration within an integrated approach. These assays can be used as part of a weight of evidence evaluation to determine whether to consider animal testing or if a POD can be determined for risk assessment purposes without the use of animals. Each test can provide insight on one key event of the AOP, which collectively, may provide a comprehensive picture of the likelihood of toxicity.

A number of different types of *in vitro* test methods, summarized in [REF _Ref46931271 \h * MERGEFORMAT], may be used to query key events in AOPs relevant to the disruption of lung function by surfactants [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Clippinger *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA] have also described a decision tree and potential key events that can be used to design pathway-based approaches for *in vitro* testing of inhalation exposures.

Table [SEQ Table * ARABIC]. Potential Methods for Evaluating Chemicals in the Surfactant Category.

Commented [A33]: Need to add the 'Tiers' in the Scheme to

Level of Biological Organization	*Key Events*	*In Vitro Assay*	*Test System*
Molecular Initiating Events (MIEs)	Interaction with pulmonary surfactant	*In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant interaction, *e.g.*, as described by Sorli *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA], Guzman and Santini (20 Please add this reference here: Guzman, E and Santini, E. Lung surfactant-particles at fluid interfaces for toxicity assessments. Current Opinion in Collo
	Interaction with cell membrane and cell membrane components and interaction	Hemoglobin Denaturation Assay, Liposome Assay, and *In Vitro/Ex Vivo* Irritation Assays	• Hemoglobin denaturation assay, *e.g.*, as described by Hayashi *et al.* (1994) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1994</Year><RecNum>14838</RecNum><DisplayText>[95]</DisplayText><record><rec-nun id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596732926">14838</key></foreign-keys><ref-type name="Journal Article">17</ref-type> H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Kato, S.</author></authors></contributors><auth-address>Shiseido I address><titles><title>Multivariate factorial analysis of data obtained in seven in vitro test systems for predicting eye irritancy</title><secondary-title> published in association with BIBRA</alt-title></titles><periodical><full-title>Toxicology in vitro : an international journal published in association w periodical><full-title>Toxicology in vitro : an international journal published in association with BIBRA</full-title><abbr-1>Toxicol In Vitro</abbr-1> 20</pages><volume>8</volume><number>2</number><edition>1994/04/01</edition><dates><year>1994</year><pub-dates><date>Apr</date></pu num>20692908</accession-num><urls></urls><electronic-resource-num>10.1016/0887-2333(94)90185-6</electronic-resource-num><remote-databas provider><language>eng</language></record></Cite></EndNote>] • Liposome assay, *e.g.*, as described by Kapoor *et al.* (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><Rec number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300">14834</key> type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors></contributors> Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalr science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</a science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</numbe Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques, Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><keyword>Huma Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-Active Ager dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electronic-resource provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] • *In vitro/ex vivo* eye irritation tests for penetrance, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD TG 492) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[97]</DisplayText><record><rec-numb id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithelium (RhCE)

			irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-titl https://www.oecd-ilibrary.org/docserver/9789264242548- en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><volume>492</ Bovine Corneal Opacity and Permeability Test (OECD TG 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year> number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719">14802</key> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeability Test Metho Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846- en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><volume>437</ Isolated Chicken Eye Test (OECD TG 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14804</ number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></foreign-keys><ref- type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for identifying I) chei irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-titl https://www.oecd-ilibrary.org/docserver/9789264203860- en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><volume>438</vo *etc.*
Cellular Level Events (CLEs)	Loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• *In vitro/ex vivo* eye irritation tests for cytotoxicity, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD TG 492) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[97]</DisplayText><record><rec-numb id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article">17</ref- type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithelium (RhCE) irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-titl https://www.oecd-ilibrary.org/docserver/9789264242548- en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><volume>492</ Bovine Corneal Opacity and Permeability Test (OECD TG 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year> number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719">14802</key> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeability Test Metho Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846- en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><volume>437</ Isolated Chicken Eye Test (OECD TG 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14804</ number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></foreign-keys><ref- type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for identifying I) chei irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-titl https://www.oecd-ilibrary.org/docserver/9789264203860- en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><volume>438</vo *etc.*

			• Cell membrane integrity test (LDH-cytotoxicity assay), cell viability assays (*e.g.*, MTT, resazurin [ADDIN EN.CITE ADDIN EN.CITE.DATA], a membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCVAM, 2006) [ADDIN EN.CITE <EndNote><Cite><Author>ICCVAM</Author><Year>2006</Year><RecNum>14805</RecNum><DisplayText>[101]</DisplayText><record><rec-n id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044231">14805</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ICCVAM</author></authors></contributors><titles><title>In vitro Cytotoxicity Test Methods for Estimating S title>ICCVAM Test Method Evaluation Report</secondary-title></titles><periodical><full-title>ICCVAM Test Method Evaluation Report</full-title> https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf</pages><volume>NIH Publication No. 07-4519</volume><dates>
Tissue or Organ Level Events (OLEs)	Tissue level events	Human organotypic Airway Cultures	• 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (*e.g.*, EpiAirway™, MucilAir™, SmallAir™, EpiAlveolar™, *etc.*) u [ADDIN EN.CITE ADDIN EN.CITE.DATA]
	Tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test, *e.g.,* as described by Hess *et al.* (2016) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and Neuhaus *et al.* (2017, 2018

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There may be multiple ~~AOP~~MOAs that would be relevant to the Surfactant Category. The MIE for a proposed MOA ~~AOP~~ under development is the interaction of a substance with lung surfactant, which may lower the surface tension and disrupt lung surfactant function [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Sorli *et al*. (2017) [ADDIN EN.CITE ADDIN EN.CITE.DATA] developed an *in vitro* lung surfactant interaction assay that specifically measures whether a substance alters the surface tension of pulmonary surfactant. The assay was initially developed for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle that may be relevant for some types of surfactants.

The proposed MIE for another AOP relevant to surfactants is direct interaction with AEC or pulmonary cell membranes, which may be followed by cytotoxicity. While the hemoglobin denaturation and liposome assays and *in vitro* eye irritation assays do not directly measure effects on membranes of AEC, these assays have been shown to be useful screening approaches for determining the ability of surfactants to interact with cellular membrane components and cell membrane penetration. For example, Hayashi *et al.* (1995) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1995</Year><RecNum>14833</RecNum><DisplayText>[105]</DisplayText><record><rec-number>14833</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539110">14833</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hayashi, T.</author><author>Itagaki, H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Sato, Y.</author><author>Suzuki, Y.</author></authors></contributors><auth-address>Shiseido Research Center, Yokohama, Japan.</auth-address><titles><title>Hemoglobin denaturation caused by surfactants</title><secondary-title>Biol Pharm Bull</secondary-title><alt-title>Biological & pharmaceutical bulletin</alt-title></titles><alt-periodical><full-title>Biological & Pharmaceutical Bulletin</full-title><abbr-1>Biol. Pharm. Bull.</abbr-1></alt-periodical><pages>540-3</pages><volume>18</volume><number>4</number><edition>1995/04/01</edition><keywords><keyword>Chromatography, High Pressure Liquid</keyword><keyword>Circular Dichroism</keyword><keyword>Hemoglobins/*chemistry</keyword><keyword>Irritants/pharmacology</keyword><keyword>Protein Denaturation/drug effects</keyword><keyword>Sodium Dodecyl

[PAGE]

Sulfate/pharmacology</keyword><keyword>Spectrophotometry</keyword><keyword>Structur
e-Activity Relationship</keyword><keyword>Surface-Active
Agents/*pharmacology</keyword><keyword>Taurine/analogs &
derivatives/pharmacology</keyword></keywords><dates><year>1995</year><pub-
dates><date>Apr</date></pub-dates></dates><isbn>0918-6158 (Print)
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6158</isbn><accession-num>7655423</accession-num><urls></urls><electronic-resource-
num>10.1248/bpb.18.540</electronic-resource-num><remote-database-
provider>NLM</remote-database-
provider><language>eng</language></record></Cite></EndNote>] showed that charged surfactant molecules can interfere with charged side chains of the hemoglobin protein. These interactions led to disruption of the three-dimensional (3D) structure of hemoglobin, causing a change in light absorbance that can be measured. Increasing concentrations of SDS and sodium lauroylmethyltaurate (LMT; CASRN 4337-75-1) were tested in this assay and showed concentration dependent increases in hemoglobin denaturation, which correlated with irritation effects in the Draize eye test [ADDIN EN.CITE ADDIN EN.CITE.DATA].

The liposome assay can be used to assess disruption of the lipid bilayer of the membrane from interaction with surfactant chemistries. Kapoor *et al*. (2009) [ADDIN EN.CITE
<EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><RecNum>14834</RecNum
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Article">17</ref-type><contributors><authors><author>Kapoor, Y.</author><author>Howell,
B. A.</author><author>Chauhan, A.</author></authors></contributors><auth-

address>Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalmol Vis Sci</secondary-title><alt-title>Investigative ophthalmology & visual science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></periodical><alt-periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</number><edition>2009/01/27</edition><keywords><keyword>Conjunctival Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques, Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><keyword>Humans</keyword><keyword>*Liposomes</keyword><keyword>Luminescent Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-Active Agents/*toxicity</keyword></keywords><dates><year>2009</year><pub-dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electronic-resource-num>10.1167/iovs.08-2980</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] measured the release of calcein dye from liposomes following exposure to various surfactants and showed a positive correlation with these findings and data from the Draize eye test. The hemoglobin denaturation

and liposomal assays were both optimized and validated against eye irritation data; therefore, these assays may provide an opportunity to evaluate the effects of surfactants on the respiratory tract. Further *in vitro* testing of known surfactants with existing data alongside new chemical substances will help benchmark the results. Nonetheless, these assays are useful for understanding the potential toxicity of a new surfactant substance to AEC or pulmonary cell membranes.

The use of *ex vivo* eye irritation studies may provide indirect measures of surfactants on cell membranes, which may be relevant to the effects observed from comparator substances in the respiratory tract. For example, Bader *et al*. (2013) [ADDIN EN.CITE <EndNote><Cite><Author>Bader</Author><Year>2014</Year><RecNum>14807</RecNum><DisplayText>[107]</DisplayText><record><rec-number>14807</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044694">14807</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bader, J.E.</author><author>Norman, K.G.</author><author>Raabe, H.</author></authors></contributors><titles><title>Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability Assay</title><secondary-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</secondary-title></titles><periodical><full-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</full-title></periodical><pages>https://iivs.org/wp-content/uploads/2018/08/iivs_poster_predicting-ocular-irritation-of-surfactants-using-the-bovine-corneal-opacity-and-permeability-assay.pdf</pages><dates><year>2014</year></dates><urls></urls></record></Cite></EndNot

e>] reported that the Bovine Corneal Opacity and Permeability (BCOP) assay was effective at demonstrating that nonionic (*i.e.*, octylphenoxypolyethoxyethanol), anionic (*i.e.*, SDS), and cationic (*i.e.*, BAC) substances cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. The permeability score was more predictive of eye irritation than the ocular opacity score for octylphenoxypolyethoxyethanol and SDS, whereas with BAC, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation of opacity and permeability measures of surfactants tested in the BCOP may be helpful with elucidating toxicity to AEC or pulmonary cell membranes.

In addition, information on the potential of a substance to cause skin irritation (*e.g.*, OECD TG 439 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14808</RecNum><DisplayText>[108]</DisplayText><record><rec-number>14808</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044884">14808</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed Human Epidermis Test Method, In vitro Skin Irritation</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>26, https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1596045726&id=id&accname=guest&checksum=2580E92A5C8

89D0DD65599260E7866D3</pages><volume>439</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]) and/or skin corrosion (*e.g.*, OECD TG 431 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14809</RecNum><DisplayText>[109]</DisplayText><record><rec-number>14809</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044976">14809</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>29, https://www.oecd-ilibrary.org/docserver/9789264264618-en.pdf?expires=1596045820&id=id&accname=guest&checksum=E3EE55CBAAFAF0432EAD109F1B39ECF0</pages><volume>431</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]) *in vitro*, can provide supporting evidence of the potential for a substance to cause similar irritant or corrosive effects in respiratory tract cells. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, demonstrated by DDAC that causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 [ADDIN EN.CITE <EndNote><Cite><Author>Sigma-Aldrich</Author><Year>2020</Year><RecNum>14810</RecNum><DisplayText>[110]</DisplayText><record><rec-number>14810</rec-number><foreign-keys><key app="EN" db-

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Cellular Level Effects

In vitro/ex vivo assays can be used to assess ~~key events on the~~ cellular level effects ~~in AOPs relevant~~of chemicals in ~~to~~ the Surfactant Category (see Supplemental Table 1 in Clippinger *et al.*, 2018 [ADDIN EN.CITE ADDIN EN.CITE.DATA]). For general cytotoxicity ([REF _Ref46931271 \h * MERGEFORMAT]), cell lines are available that are known to be sensitive to the effects of surfactants. Use of the BALB/c 3T3 NRU cytotoxicity test to reduce animal testing by estimating starting doses for acute oral toxicity testing has been reviewed and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and is an OECD guidance document [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The surfactants with known inhalation toxicity (*e.g.*, octylphenoxypolyethoxyethanol, oleoyl sarcosine, DDAC, or BAC) should be tested in parallel with the new chemical substance to benchmark the results, thereby providing reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

Tissue or Organ Level Effects

Based on the results of testing cellular level key events, it may be necessary to perform additional testing. Human and animal airway epithelia are composed of multiple cell types that each have specialized functions, making the use of 3D co-culture assays more physiologically relevant than 2D monoculture systems. Thus, several human organotypic airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems. Two commonly employed systems are EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively.

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Organotypic airway cultures, such as EpiAirway™ and MucilAir™, [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[112]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33,

https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain homeostasis for months in culture. Because of these characteristics, these human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Dosimetry models such as the RDDR or MPPD can be used to predict the anatomical area and internal amounts delivered in various regions of the respiratory system for humans under the target inhalation exposure scenario for the given use case. Different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. MucilAir™ provides a 3D co-culture model of cells from nasal, tracheal or bronchial sites, and SmallAir™ provides a co-culture model of cells from small airways. EpiAirway™ is composed of a co-culture of normal human tracheal/bronchial epithelial cells, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts (available with and without macrophages).

Exposure of respiratory tract 3D co-culture models to aerosols at the air liquid interface (ALI) using an *in vitro* exposure system, such as those available from Vitrocell® Systems, provides an exposure more comparable to real-life scenarios for inhaled aerosols. The tradeoff has been a lower throughput compared to *in vitro* two-dimensional exposure systems; however, 3D tissue models and ALI exposure systems are now available in a 96-well format. Dilution in medium

and interaction with medium components does not occur in the ALI exposure systems as in submerged culture systems. The respiratory tract 3D co-culture models are more physiologically relevant because there is an interaction of the aerosol with a mucus or surfactant layer, as in humans.

Exposures of these organotypic cultures at the ALI can be combined with other assays for assessing cell function and viability in an AOP approach. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays (such as MTT, resazurin, or ATP assays), have all been reported for use with these cultures. Further, multiple assays can be performed on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant octylphenoxypolyethoxyethanol at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, provided dosimetry models are available for translation of the internal dose achieved under culture conditions to an equivalent inhalation exposure for the human scenario of interest. Examples of *in vitro* dosimetry models to predict particle doses for submerged cell culture include the *In vitro* Sedimentation, Diffusion and Dosimetry model (ISDD) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and the *In vitro* Sedimentation, Diffusion and Dissolution Dosimetry (ISD3) model [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Significant progress has been made toward achieving the objectives to use high-throughput *in vitro* assays and computational models to evaluate potential adverse effects of chemical exposures [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[16, 115]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite><Cite><Author>NRC</Author><Year>2017</Year><RecNum>14812</RecNum><record><rec-number>14812</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045703">14812</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Using 21st Century Science to Improve Risk-Related Evaluations, Washington, D.C., The National Academies Press</title></titles><pages>200, https://doi.org/10.17226/24635</pages><volume>ISBNs: Ebook: 978-0-309-45351-6; Paperback: 978-0-309-45348-

6</volume><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. To translate the effects to higher levels of biological organization, a battery of assays with varying complexity and physiological relevance may be needed. The 3D human organotypic airway cultures add evidence to an AOP approach and increase confidence in the physiological relevance to humans.

Precision-cut lung slices (PCLS) provide an additional method to develop key event data using *ex vivo* cultures of human or rodent lung slices. The PCLS can be used to measure multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo*. PCLS are often used in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema [ADDIN EN.CITE <EndNote><Cite><Author>Sanderson</Author><Year>2011</Year><RecNum>14814</RecNum><DisplayText>[117]</DisplayText><record><rec-number>14814</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046031">14814</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sanderson, M. J.</author></authors></contributors><auth-address>Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA 01655, USA. Michael.Sanderson@umassmed.edu</auth-address><titles><title>Exploring lung

physiology in health and disease with lung slices</title><secondary-title>Pulm Pharmacol Ther</secondary-title><alt-title>Pulmonary pharmacology & therapeutics</alt-title></titles><periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></periodical><alt-periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></alt-periodical><pages>452-65</pages><volume>24</volume><number>5</number><edition>2011/05/24</edition><keywords><keyword>Animals</keyword><keyword>Cell Physiological Phenomena</keyword><keyword>Disease Models, Animal</keyword><keyword>Humans</keyword><keyword>Lung/pathology/*physiology</keyword><keyword>Lung Diseases/*pathology</keyword><keyword>Microscopy/methods</keyword><keyword>Muscle Contraction/physiology</keyword><keyword>Organ Culture Techniques</keyword></keywords><dates><year>2011</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1094-5539 (Print)
1094-5539</isbn><accession-num>21600999</accession-num><urls></urls><custom2>PMC3168687</custom2><custom6>NIHMS296121</custom6><electronic-resource-num>10.1016/j.pupt.2011.05.001</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be evaluated. One further advantage of PCLS is that the assay can be performed on multiple species to determine inter-species variability in susceptibility.

Human PCLS, derived from, for example, rejected but otherwise healthy transplant tissue, can be used to measure cell/tissue viability, local respiratory inflammation, and physiological function. These endpoints can be measured in single and repeated exposures in a metabolically competent system within the normal architecture of the lung in a more relevant model system, replacing the need for animal testing [ADDIN EN.CITE ADDIN EN.CITE.DATA].

When human PCLS are not available, rat PCLS provide an alternate option. The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed correlation with *in vivo* LC_{50} values [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The use of rat PCLS reduce the number of animals used to conduct dose response studies, as compared to *in vivo* inhalation tests. From a rat lung (1 g), approximately 200 slices can be prepared. In general, for each test substance concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested using tissue from a single rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures *via* liquid media or, with additional adaptations, air. As such, rodent PCLS meet the goal of reducing animal testing, although dosimetry models for their translation to HEC are not yet developed. Mechanistic rodent and human PCLS studies may be conducted in parallel to understand species specific difference in toxicological effects. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations of an ~~AOP~~ MOA Approach to the Surfactant Category

A number of *in vitro* assays have been discussed as to their potential utility for assessing key events in an AOP(s) relevant to characterize the Surfactant Category. Uncertainties and limitations associated with these assays are discussed for each of the above testing systems, as well as others [ADDIN EN.CITE ADDIN EN.CITE.DATA]. It is important to consider that these assays were not systematically tested using surfactants. Nonetheless, these assays can be conducted using ~~an AOP~~ MOA approach to provide information on whether a new chemical meets the Surfactant Category criteria and/or to understand whether the new chemical may be more or less bioactive or toxic than the sub-category comparator chemicals. EPA will generally use the framework and analogue toxicity data identified in this investigation to assess potential risks from surfactants.

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In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment continues to evolve. A fit-for-purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, and domain of applicability for evaluating a new method to inform specific decisions has emerged from the regulatory science community to address the challenges posed for validation of NAMs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Such fit-for-purpose validation approaches are intended to be flexible and adaptable and to provide data sets, prediction analysis results, inference models, *etc*. in a transparent manner that enable other scientists to confirm the performance of the assays and inference models, as well as evaluate the rationale for using these assays in a specific decision context.

Once such fit-for-purpose scientific evaluations are documented, there are several ways that these assays can be used to reduce and replace animal testing. First, testing can be performed based on an AOP approach to evaluate the potency of new surfactants versus a comparator substance within the relevant subcategory that has repeated exposure inhalation toxicity data. Second, depositional data using models such as the RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

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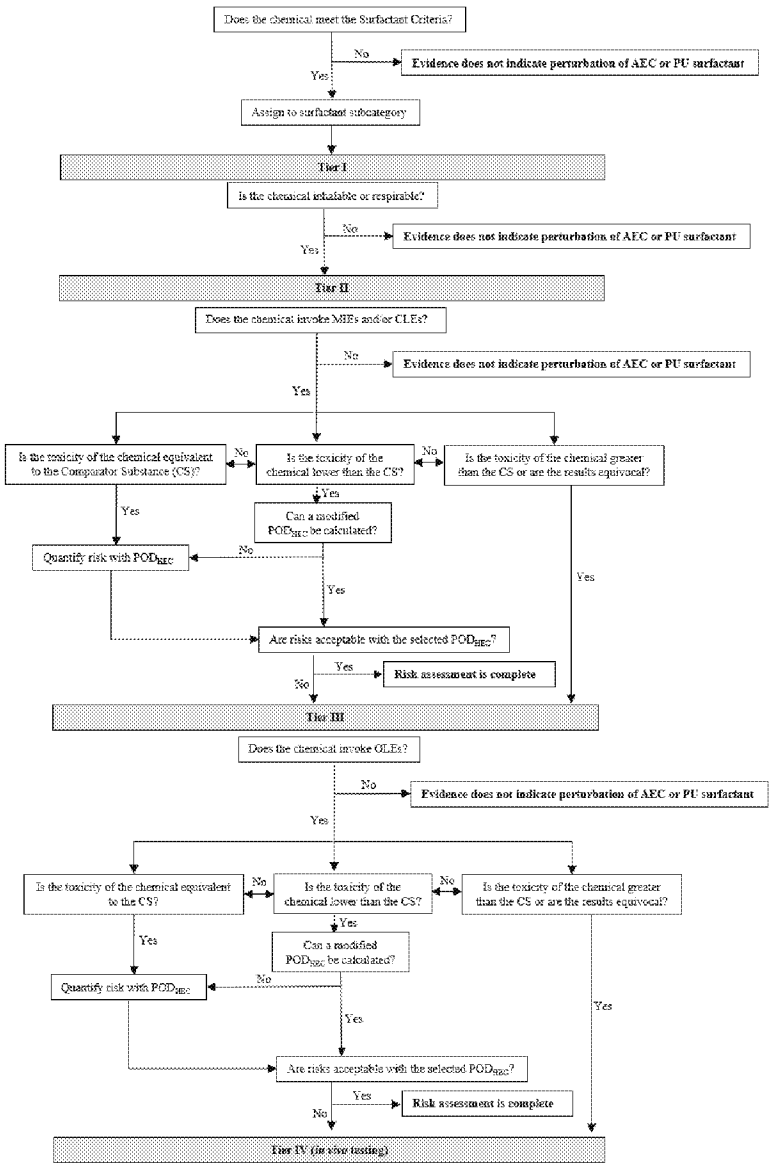
Tiered-testing Strategy

The first step in the tiered-testing strategy is to determine if the evaluated substance meets the Surfactant Criteria. If so, then assign the substance to the appropriate surfactant subcategory (nonionic, anionic, or cationic) and determine whether any of the representative subcategory chemicals may serve as an acceptable toxicological analogue for risk assessment or as a comparator substance for tiered testing. If a representative subcategory chemical is determined to be an acceptable toxicological analogue to the new chemical substance, then quantify risks using the toxicological analogue. If the MOE is equal to or greater than the benchmark MOE, then tiered testing is not required on the new chemical substance. If the MOE is lower than the benchmark MOE or if a determination cannot be made on whether any of the representative subcategory chemicals are acceptable toxicological analogues, then proceed with tiered testing using the most appropriate subcategory chemical as a comparator substance to the new chemical substance. As detailed below, the tiered-testing strategy commences with the least complex, most efficient testing methods, and at each subsequent tier, the complexity of the test system

increases, commensurate with key events in proposed AOPs relevant to the Surfactant Category, to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system. It is envisioned that both the new chemical substance and the comparator substance will be evaluated side-by-side in the NAM assays. The results of these studies may lead to the conclusion that the comparator substance is an acceptable toxicological analogue to the new chemical substance. Alternatively, the results may support that higher tiered testing is warranted, particularly when the new chemical substance has higher toxicity than the comparator substance. If *in vivo* testing is conducted, it may not be necessary to run the comparator substance in the *in vivo* tests, given that suitable inhalation studies are available on the comparator substances. A summary of the proposed tiered-testing strategy is provided in [REF _Ref48210489 \h * MERGEFORMAT] and discussed further below.



Scheme [SEQ Scheme * ARABIC]. Proposed tiered-testing strategy for general surfactants.

Commented [A36]: Are we 'revisting' the Scheme for Surfactants, i.e. like for Insoluble Polymers?

Add a "Legend' to the Scheme

[PAGE]

Tier I—Physicochemical properties

Surfactants are proposed to cause a specific sequence of biological events in the respiratory tract if they are inhaled. Manufacture, processing, or use of a surfactant in an inhalable form, (*i.e.*, ≤ 100 μm aerodynamic diameter) is therefore, an initial consideration of the potential for a surfactant to cause toxicity to the respiratory tract. Particle size is an established parameter for determining inhalability/respirability of particles/droplets. Several validated test methods exist for determining potential inhalability/respirability, *i.e.*, particle size, of a new chemical substance (*e.g.*, OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><d

ates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], ISO 21501-1:2009 [ADDIN EN.CITE <EndNote><Cite><Author>ISO</Author><Year>2009</Year><RecNum>14820</RecNum><DisplayText>[121]</DisplayText><record><rec-number>14820</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046993">14820</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ISO</author></authors></contributors><titles><title>Determination of particle size distribution — Single particle light interaction methods — Part 1: Light scattering aerosol spectrometer</title></titles><pages>https://www.iso.org/standard/42728.html</pages><volume>ISO 21501-1:2009</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], OECD TG 110 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>1981</Year><RecNum>14821</RecNum><DisplayText>[122]</DisplayText><record><rec-number>14821</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047114">14821</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Particle Size Distribution/Fibre Length and Diameter Distributions; Method A: Particle Size Distribution (effective hydrodynamic radius); Method B: Fibre Length and Diameter Distributions</title><secondary-title>OECD Guidelines for the Testing of

Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>13, https://www.oecd-ilibrary.org/docserver/9789264069688-en.pdf?expires=1596047951&id=id&accname=guest&checksum=A9C13F0DFDCF2A5DD4DD39DAC64C47BC</pages><volume>110</volume><dates><year>1981</year></dates><urls></urls></record></Cite></EndNote>], and OPPTS 830.7520 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>14822</RecNum><DisplayText>[123]</DisplayText><record><rec-number>14822</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047315">14822</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Particle Size, Fiber Length, and Diameter Distribution</title><secondary-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</secondary-title></titles><periodical><full-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPPT-2009-0151-0030&contentType=pdf</pages><volume>EPA 712-C-96-037</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]). The studies shown in Table 3 suggest that the total respiratory tract may be affected from surfactants; therefore, inhalable forms ($\leq$ 100 μm) were identified as the most relevant for quantitative inhalation risk assessment. As a practical matter, a particle size cutoff of greater than 1% inhalable particles/droplets by weight (wt%), determined in a well conducted study using a

valid measurement method will generally be considered as triggering a quantitative assessment of inhalation toxicity on a new chemical substance meeting the Surfactant Criteria. EPA will generally assess the potential inhalation toxicity for a new surfactant chemical substance when the manufacture, processing or use results in greater than 1% (by weight) of the surfactant particles/droplets having a particle size of less than 100 μm. This wt% cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2017</Year><RecNum>14823</RecNum><DisplayText>[124]</DisplayText><record><rec-number>14823</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047488">14823</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>3641-3655</pages><volume>82</volume><number>8</number><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>].

If inhalable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information to determine whether a new chemical substance invokes MIEs and cellular level key events. In order to determine the best approach for *in vitro*/*ex vivo* testing, a pre-notice consultation with EPA is highly encouraged. In general, the testing approach in this tier should include a combination of assays, such as one that measures epithelial lining fluid/cell perturbation or pulmonary surfactant interaction/loss of function, one that measures cell membrane interaction/disruption/penetration), and one that measures loss of barrier integrity or general cytotoxicity (see [REF _Ref46931271 \h * MERGEFORMAT]). *In vitro*/*ex vivo* eye irritation studies may also be used to demonstrate cell interaction or penetration and general cytotoxicity, and *in vitro* skin irritation/corrosion studies can provide supporting evidence of possible irritant or corrosive effects in the respiratory tract.

For each assay, the comparator substance for the respective subcategory of surfactants should be tested under identical conditions. Further, the particle size distribution data may be used with dosimetry models such as RDDR or MPPD to aid with identifying the regions in the respiratory tract where deposition is expected to occur and the appropriate test concentrations for the *in vitro*/*ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc*.

Notwithstanding the uncertainties with the above assays, each may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE) for the purpose of evaluating the relative potency of the new chemical substance versus the comparator substance. Several investigations have provided insight on approaches for

accomplishing this, although with different assay systems [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In doing so, a weight of scientific evidence evaluation should be performed considering the structural features, physicochemical properties, and assay results on the new chemical substance versus the comparator substance. Based on this evaluation, the most biologically relevant endpoint(s) should be used to calculate a POD. BMD modeling may be applied to derive a $BMCL_{1SD}$ metric, as a possible metric, although the metric of one standard deviation should be used with caution [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2019</Year><RecNum>14825</RecNum><DisplayText>[126]</DisplayText><record><rec-number>14825</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048386">14825</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Transmittal of Meeting Minutes and Final Report for the Federal Insecticide Fungicide and Rodenticide Act, Science Advisory Panel (FIFRA SAP) Meeting held on December 4 and 6, 2018</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>51,https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2018-0517-0030&contentType=pdf</pages><volume>EPA-HQ-OPP-2018-0517</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] . Alternative metrics should be considered, as our understanding evolves for various *in vitro*

assays and endpoints. For example, the pharmaceutical industry has used fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc.*) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Regardless of the metric used, a justification for its selection should be provided. In those situations where data are not amenable to BMD modeling, the *in vitro* concentration tested should be determined based on the expected HEC for the appropriate subcategory (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC.

Given that the understanding of IVIVE is evolving, assay results should be interpreted in a manner consistent with the weight of scientific evidence, as noted above, while recognizing that uncertainties are often dealt with by erring on the side of conservativism. Therefore, the following initial default criteria are proposed for utilizing the assay results, and when possible, the IVIVE estimates. These criteria are consistent with EPA's approach for evaluating non-animal skin sensitization data [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14832</RecNum><DisplayText>[128]</DisplayText><record><rec-number>14832</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596244984">14832</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement for Laboratory Animal Testing (draft for public comment: April 4, 2018)</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and

Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2016-0093-0090&contentType=pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], while recognizing that the weight of scientific evidence may support an alternative interpretation to the default criteria.

The Tier II assays evaluate biologically relevant endpoints representing key events in AOPs relevant to the Surfactant Category. The results of the comparator substance and the new chemical substance in these assays provide a basis for evaluating the suitability of using the comparator substance to evaluate toxicity of the new chemical substance. Consideration should also be given to differences in the specific physicochemical properties influencing inhaled deposition (*i.e.*, MMAD, GSD, and density) between the comparator substance the new chemical. Dosimetry models such as RDDR and MPPD can be used to inform these comparisons.

If comparable toxicity is observed between the comparator substance and the new chemical substance in the Tier II assays, the POD_{HEC} from the comparator substance may be appropriately used as a toxicological analogue for quantifying the MOE. If calculated risk is acceptable stop at Tier II, otherwise proceed to Tier III.

If lower toxicity is observed for the new chemical substance versus the comparator substance in the Tier II assays, then these data should be used to determine if a modified POD_{HEC} can be quantified for the new chemical substance. If this is possible, the modified POD_{HEC} for the new chemical substance should be used for quantifying the MOE. If calculated risk is acceptable, then stop at Tier II. However, if it is not possible to calculate a modified POD_{HEC}, then the comparator substance POD_{HEC} could be used as a worse-case toxicological analogue for risk assessment. If no acceptable risk can be calculated, proceed to Tier III.

If greater toxicity is observed with the new chemical substance versus the comparator substance in the Tier II assays, suggesting risks would be identified as unacceptable, proceed to Tier III. Alternatively, there may be scientifically justified reasons for an alternative interpretation, which should be clearly articulated with the weight of scientific evidence evaluation. Otherwise, it may be necessary to proceed to Tier III.

If the results from the Tier II assays are equivocal (*i.e.*, they do not demonstrate comparable or lower toxicity of the new chemical substance versus the comparator substance), and there is no clear rationale or explanation, then proceed to Tier III testing because the data are too uncertain to make a reasoned evaluation on the potential health risks, following potential inhalation exposures.

Tier III – 3D Human Airway Models/PCLS Assay

Several testing options are available for evaluating tissue and organ level key events in an AOP relevant to the Surfactant Category. The test system employed should focus on evaluating effects

in the respiratory tract at the predicted sites of deposition (*e.g.*, ET, TB and/or PU regions), based on the particle size distribution data generated under Tier I and using RDDR or MPPD modeling. A justification for using a system(s) should be provided and may be discussed with EPA as part of a pre-notice consultation. Representative test systems include those listed in [REF _Ref46931271 \h * MERGEFORMAT].

Based on the results of the 3D-construct and/or PCLS testing, IVIVE may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[112]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM) </title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-

title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33, https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use or if the Tier III test data are not amenable for developing a POD_{HEC}, then proceed to Tier IV.

Tier IV – *In vivo* studies

Strategic *in vivo* testing may be considered as a last resort to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the comparator substances in a subcategory has representative toxicological properties to the new chemical substance, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving modified POD_{HEC}s. A pre-notice consultation meeting with EPA is strongly encouraged prior to initiating any vertebrate animal testing. This point is especially important because TSCA section 4(h)(3) indicates that any person developing information for submission under TSCA section 5 on a voluntary basis shall first attempt to develop the

information by means of an alternative test method or strategy identified by EPA before conducting new vertebrate animal testing [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[85]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>].

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with epithelial lining fluid/pulmonary surfactant and/or disrupting cellular membranes and epithelial cytotoxicity. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests should be considered:

- Step 1: OECD TGs 433, 436, and 403 address acute inhalation toxicity testing. OECD TG 433 is based on evident clinical signs of toxicity rather than death as an endpoint

(refinement) and TG 436 uses fewer of animals (reduction), and therefore, they should be considered before TG 403. Any protocol modifications should be discussed with EPA during a pre-notice consultation meeting.**

- Step 2: 5-Day inhalation study with a 14-day observation period** to address progression/resolution of effects. The OECD TG 412 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14828</RecNum><DisplayText>[129]</DisplayText><record><rec-number>14828</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048957">14828</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>28-day (subacute) inhalation toxicity study</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>23, https://doi.org/10.1787/9789264070783-en</pages><volume>412</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be used, but the exposure duration should be 5 days.

**Modifications may include pulmonary function testing (if measurable), analysis of BALF, LDH release, complete histopathological analysis of the respiratory tract and blood oxygen (pO_2) content. OECD TG 412 and OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum>

<DisplayText>[79]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>2001</Year><RecNum>14826</RecNum><DisplayText>[130]</DisplayText><record><rec-number>14826</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048712">14826</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Alarie, Y.</author><author>Nielsen, G.D.</author><author>Schaper, M.M.</author></authors><secondary-

authors><author>Spengler, B.</author><author>Samet, J. M.</author><author>McCarthy, J.F.</author></secondary-authors></contributors><titles><title>Animal Bioassays for Evaluation of Indoor Air Quality</title><secondary-title>Indoor Air Quality Handbook</secondary-title></titles><pages>23.21-23.49.</pages><dates><year>2001</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill</publisher><urls></urls></record></Cite></EndNote>].

The results of the *in vivo* testing should be used for reassessing and recharacterizing the risks of the new chemical substance.

CONCLUSIONS

The overall objective of this investigation was to develop a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. This investigation developed physical-chemical properties, *i.e.*, the Surfactant Criteria, assessors and product stewards can use for determining whether a new chemical substance can be considered a surfactant. Further, properties and characteristics are provided to divide the Surfactant Category into sub-categories for nonionic, anionic, and cationic surfactants, which is important from a toxicological perspective. A systematic literature search and review were conducted to identify data to define a Surfactant Category and substances from which PODs were identified from inhalation toxicity studies. To facilitate chemical comparisons, animal toxicity studies that could be used to derive PODs for risk assessments were identified for at least one chemical substance for each sub-category and converted to HECs using established methods developed by EPA.

Finally, a tiered-testing strategy for generating *de novo* data for new surfactant substances is provided that integrates a variety of currently available NAMs using an AOP framework. The use of this tiered-testing strategy will inform the available data on surfactants and provide greater confidence in the use of non-vertebrate testing approaches for assessing the potential risks of new chemical substances. It also offers advantages to regulators, the regulated community, and consumers because: 1) integrating NAMs into a category testing approach supports EPA, TSCA and product stewardship goals of reducing and replacing vertebrate animal testing; 2) decision analysis for higher tiered testing takes into consideration mechanistic responses, dosimetry, and exposure information; and 3) it encourages development of mechanistic data to advance the understanding of the potential inhalation toxicity of surfactants, which will drive the development of newer and safer chemistries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information file contains the following:

Section 1. Systematic Literature Review

Section 2. RDDR Modeling Outputs

AUTHOR INFORMATION

Corresponding Author

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

Funding Sources

EPA sponsored the initial literature review through a government contract to SRC (68HERH19F0197 (TO#07)). The American Chemistry Council's TSCA Section 5 Testing Consortium sponsored an updated literature review by an independent third party.

Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

Disclosures: TS, AMJ, KS, WI, and TRH are employed by the federal government. MPH, WK, AMK, SM, LJ, JLR, AT, and RT are employed by companies that manufacture, process, and/or use surfactants. RAB and SOS are employed by a company that represents companies that manufacture, process, and/or use surfactants. PDM and SDS work for a company that received contract funding from companies that manufacture, process, and/or use surfactants. MO and JM

work for a company that receives contract funding from the federal government. AJC and MS are employed by a company whose mission is to advance animal-free testing approaches that protect human health and the environment.

REFERENCES

[ADDIN EN.REFLIST]

The reviews for your paper are enclosed with this letter. Please consider the reviewers' comments. They have raised points that require significant consideration and revision of the manuscript before it is suitable for publication.

In addition, the authors should clarify the message of their work and decrease its length: many materials should be included as a supplementary material.

As the manuscript is considered for the Special Issue: Computational Toxicology (January 2021), we needed to shorten the usual revision time. The manuscript is due 1-Dec-2020.
Please let us know if that would be convenient for you.

Please also note that all manuscripts that deserve publication and are not submitted in time for the special issue, will be published in CRT regular issue.

When submitting your revised manuscript through ACS Paragon Plus, you will be able to respond to the comments made by the reviewer(s) in the text box provided or by attaching a file containing your response letter. In your cover letter please include your detailed responses to all of the points raised by the reviewers.

When submitting a revised manuscript, authors should include a version of the manuscript that has the Tracked Changes feature turned on, so editors and reviewers can see the revisions that were made to the original manuscript. Please upload this version of the manuscript under the tag "Supporting Information for Review Only." Authors should still indicate page and line numbers when referring to edited text in their response letter to the reviewers. An unmarked, final version of the manuscript should be uploaded under the tag "Manuscript File."

Reviewer(s)' Comments to Author:
Comment Addressed – barring any objections/edits
Comment Needs Addressing – who can address?
To Do/Check in FINAL Review

Commented [TH1]: All review/edit and return to all, esp Tala by COB Th, 11/19/20

Reviewer: 1
Comments:
The work by Henry et al. presents an analysis of the impact of surfactants upon inhalation. The topic may be interesting, however authors present the topic in such a way that is very difficult to understand the message. The work deals mainly with literature data. However, the connection between them and their selection are not clear.
Response: The authors note that the comments regarding understandability are in sharp contrast to those of reviewer #2, who was complementary regarding purpose, clarity and flow. As noted by Reviewer #2, the manuscript describes "the development of a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. The paper describes in detail the literature found on surfactants and inhalation, suggest how AOPs and NAMs can be used for risk assessment, and a scheme for how this would done."

The authors have shortened the abstract and the manuscript considerably and believe this should make clearer to the reader that this is, by design, a literature-based investigation for the purpose of establishing a TSCA New Chemicals Category, for use in evaluating new surfactant chemicals under the U.S. Toxic Substances Control Act.

Some points to address are:

1. Authors should correct spelling and typos within the entire manuscript.
   Response: The authors conducted a spell check of the Draft Proof (pdf file) and of the submitted MSWord file version of the manuscript (from which the pdf was generated) and did not identify any spelling errors in either document. We cannot check on specific instances since they are not identified by the reviewer. Nonetheless, the authors have conducted a spelling and grammar check (using MSWord) on the revised manuscript and have corrected the two typographical errors specifically identified by reviewer 2.

2. Abstract is too long, and difficult to follow. Authors should rewrite concisely the abstract. They shoul present the main aims and interests of their work, avoiding to present a detailed summary.
   Response: The authors have shortened the abstract. The revised abstract is 293 words, under the journal limit of 300 words.

3. What is the meaning of EPA? Authors should define all the abbreviation.
   Response: As defined on line 28 of the Draft Proof, EPA is the abbreviation for the U.S. Environmental Protection Agency.

4. Some information about the differences between the chosen approaches, and the information contained with REACH would be required
   Response: As indicated in the draft manuscript, the approach described is focused on assessments conducted under the U.S.'s Toxic Substances Control Act. Therefore, the authors respectfully disagree that a comparison to REACH approaches is required. However, the authors have included text acknowledging REACH and to clarify that while the approaches in the manuscript are broadly

Commented [HT2]: FROM RICK BECKER:
We'll probably have to write a note to the editor pointing out that Reviewer 1's perspectives are REACH-centric, and as clearly indicated, this paper is focused on TSCA. And that while we have addressed Reviewer 1's comments on other aspects of the manuscript, we have not broadened it to address REACH, since that is not the focus or scope of our paper. And that although the underlying scientific approaches in this article are broadly applicable, a wholly separate effort would be needed to address the specific legislative and regulatory structures of REACH.

2. I suggest we add some text acknowledging REACH, but pointing out that TSCA legislation / regulations are quite different from REACH, making it even clearer (although I think it is already clear) that this approach has been developed for TSCA, and add text along the lines of "although the underlying scientific approaches in this article are broadly applicable, a wholly separate effort would be needed to address the specific legislative and regulatory structures of REACH."

applicable for assessment of surfactants, they may or may not be applicable within the specific regulatory framework of REACH.

Furthermore, both reviewers have indicated the manuscript should be shortened. To add additional information regarding REACH would be counter to this request by the reviewers.
[Discussed and decided to also write to Editor only regarding conflicting comments regarding the manuscript being too long vs adding additional information and that REACH is really out of scope of this manuscript. ...see Rick Becker notes in the Comment Bubble]

5. "A surfactant is a substance that reduces the surface tension of a liquid in which is dissolved" This definition is misleading and simple. In some cases surfactant are insoluble, eg. lipids
Response: The authors agree the definition is simple, as a starting point. It is followed by a more functional definition, i.e., regarding surface tension. Furthermore, the statement is not about water solubility and surfactants must have solubility in the solution to which they are added in order to be surface active. Nonetheless, the authors have provided an alternative definition and a reference for it in the revised manuscript, as follows:

   Any compound that reduces surface tension when dissolved in water or water solutions, or which reduces interfacial tension between two liquids, or between a liquid and a solid. *Hawley's Condensed Chemical Dictionary, R. Lewis, Van Nostrand Reinhold Co.; 1993, pg. 1108.*

6. About toxicity of surfactants is should be included in the reference list Colloids and Surfaces B: Biointerfaces 123 (2014) 701-709 [Guzman et al., 2014; on CTAB 'a cationic surfactant', i.e., quaternary ammonium cmpd]
Response: The study referenced, Guzman et al., 2014, is a study of the "The effect of a cationic surfactant, hexadecyltrimethylammonium bromide (CTAB), on the interfacial properties of seawater" and concludes, "The results of this study underline the important role of the sea organic content in enhancing the surface-activity of surfactants, which is relevant for a deeper understand of the direct and indirect effects of these types of pollutants on the physico-chemical environment in the sea coastal areas and develop mitigation strategies." This study does not measure human or mammalian surfactant toxicity; hence, the authors do not see the linkage of this reference in the discussion of toxic effects of surfactants in conducting human health risk assessments under TSCA.

7. About inhalation toxicity, it should be included the work Current Opinion in Colloid and Interface Science 39 (2019) 24-39 [Guzman & Santini; Review article focusing on effects of particulates on biological/lung surfactants; has some good words about use of model systems to evaluate effects on lung surfactants]
[redacted] [Rick] The reference, Guzman & Santini (Current Opinion in Colloid and Interface Science 39 (2019) 24-39) discusses model systems to evaluate effects of particulates on lung surfactants. Although this article deals with particulates, it describes the use of model systems to evaluate effects on lung surfactants. Therefore, this reference was added to Table 4, Potential Methods for Evaluating Chemicals in the Surfactant Category, in the row "Interaction with pulmonary surfactant."

8. Authors should provide a definition of toxicokinetic and toxicodynamic -
Response: Toxicokinetics is generally considered the processes of absorption, distribution, metabolism, and excretion of a toxicant. Toxicodynamics is generally considered the mode of toxic action of a toxicant. The authors have provided these general definitions parenthetically at first occurrence of these terms in the revised manuscript (i.e., page XX INTRO)

9. About the change of mechanical properties of the lung surfactant layer, the reference The Journal of Physical Chemistry C 119 (2015) 26937-26947
   Response: The reference, Guzman et al., 2015, looks at the effects of carbon nanoparticles on the primary lung surfactant, Dipalmitoylphosphatidylcholine. Carbon nanoparticles do not meet the traditional definition of a surfactant and are outside the boundary conditions in this paper which covers commercial surfactants. The authors note that there is a companion paper in this special issue of Chem Res Tox that looks at the effects of poorly soluble polymeric particles in the lungs.

10. The interest of the problem of surfactant inhalation is not clear. Normally, they are used in solution. Are the authors discussing the inhalation of the powder surfactant? Otherwise the interest of the work is scarce. This point should be clarified.
    Response: The majority of commercial surfactants are liquids and those that are powders are typically engineered to be of a non-respirable particle size. While we agree there is not significant consumer exposure via inhalation, surfactants are both manufactured and processed in industrial settings where exposure could be relevant. Additionally, there could be workplace exposure in commercial applications. EPA has the responsibility to assess these uses, which this manuscript addresses.

11. Authors mentions the subcategories of surfactants. However, they do not comment anything on the polymeric surfactant and the possible role of colloidal particles as surfactants.
    Response: The most common classifications of surfactants are based on charge. These are nonionic, anionic, cationic, and amphoteric. Polymeric surfactants are included within each of these categories based on their charge or lack thereof. Most polymeric surfactants are nonionic. As an example, the nonionic surfactant Triton X-100 (ethoxylated octylphenol) is a polymeric surfactant.

    This manuscript is focused on US EPA guidance related to the review of new chemical substances which are surfactants. Hence, colloidal particles are out of scope for this paper as they are not within the boundary criteria, nor would a colloidal suspension (e.g., a product containing particles suspended in another chemical) be subject to new chemical review.

    Commented [HT3]: Discuss with Todd

12. Authors mention several test to evaluate the toxicity. However, they do not provide details on the foundation of such tests, making difficult the comparison of data.
    Response: In the manuscript section entitled, **Use of New Approach Methods (NAMS) and *In* Vitro Testing Strategies to Reduce or Replace Vertebrate Testing**, the authors first provide a summary table (Table 4) of potential methods for evaluating chemicals in the surfactant category. This summary table is then followed by descriptions of the scientific tenets of each of the tests referenced (e.g., On page X, "Sorli et al., (2017) developed an *in vitro* lung surfactant interaction assay that specifically measures whether a substance alters the surface tension of pulmonary surfactant." and on page Y, "...several human organotypic airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems." Additionally, specific details regarding some of the most recent or novel tests are also provided (e.g., on page X, "Organotypic airway cultures, such as EpiAirway™ and MucilAir™, take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain homeostasis for months in culture. All of the tests included in the summary table and the text have been published, either as journal articles or as test guidelines by international authorities and are fully referenced. To include additional methodological details about every referenced test

would be unusual and would also be contrary to the reviewers' requests for shortening the manuscript.

Reviewer: 2

Comments:
Henry et al investigated the development of a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. The paper describes in detail the literature found on surfactants and inhalation, suggest how AOPs and NAMs can be used for risk assessment, and a scheme for how this would done.

1. **[LENGTH]** The paper is well laid out, contains clear language and flows nicely. It describes very well how the risk assessment could be performed. However, I think the length could deter some readers, if the length is kept, I would suggest that you start with an index so that readers can find any information that they are most interested in.
   Response: The authors thank the reviewer for the complements on writing and flow. In response to the reviewer's comment regarding length of the manuscript, the authors have streamlined the paper significantly [XX pages and moved certain sections (e.g., YYY) into supplementary materials.

2. **[ABBREVIATIONS]** In line with this, the paper is very abbreviation heavy, so a list of abbreviations would be very helpful, this would also let you catch those abbreviations that are not defined (at least I could not find them). These include: BMCL (p.17) UFH, UFA, and UFL (p. 68).
   Response: The authors thank the reviewer for the suggestion to make a list of abbreviations; it was most helpful in conducting final review of the manuscript. The authors suggest including the list in supplementary materials due to concerns about the length of the manuscript but will defer to the Editor regarding final placement of the list. [Tala or Keith]

3. **[FONT]** It may be a product of conversion to pdf, but the font and size changes throughout the paper.
   Response: The font type and size has been checked and made consistent throughout the document. [Tala – Final Review]

4. **[REFERENCE FORMAT]** For the references section: Several references are inconsistent in order, as they are "Surname, intials, initals, surname"
   Response: All references have been checked and comply with the format requested by the journal. [Todd/Endnote]

   e.g. 54. Alarie, Y. and M.F. Stock, Respiratory Irritancy on a Mixture containing Polyethylene Glycol Mono(Octyl)Phenyl Eether CAS #9035-19-5. ChemView - U.S. Environmental Protection Agency, 1992: p. 37,
   Response: [EPA will do]

   - Also referencing to dossiers is strange (what
   is the R?):
   59. Dossier, R., N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC

number: 701-177-3, Skin irritation/corrosion. European Chemicals Agency, 2020:
- The links in references 89 and 90 do not work (I tried 2 different browsers, at separate days)
Response: [EPA will do]

5. **For page 29**: The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less.
How is this tested?
Response: [PAGE 18 of WORD document: add (as determined by standard methods)
There are a number of techniques that utilize light scattering or steady-state fluorescence quenching to determine the formation of micelles and vesicles. The critical micelle concentration (CMC) value can be determined by plotting a curve of concentration versus surface tension. Since there are a variety of methods available, the authors did not want to suggest a specific method in order to provide flexibility to the reader.

6. **For page 75:** You write "including validated OECD methods for in vitro irritation testing and in vitro methods to specifically assess respiratory toxicity". This is not referenced, to my knowledge there are no validated OECD methods to specifically assess respiratory toxicity, please provide more info/reference.
[redacted] [EPA will do]

7. **For page 54:** Cationic Surfactants
You may like to include the paper "Airway Effects of Inhaled Quaternary Ammonium Compounds in Mice" by Larsen et al that describes airway effects after inhalation. Doi: 10.1111/j.1742-7843.2011.00851.x [ACUTE inhalation study of 4 Quats; relative potency in causing decreased tidal volume and increased respiratory rate indicating pulmonary irritation; pulmonary inflammation apparent from BAL; ADD TO IN VIVO SECTION OF CATIONIC – STARTS PAGE 34 OF WORD Document]
Response: The authors have added a summary and refence to the study by Larsen et al. to the Hazard Identification section on Cationic Surfactants. Thank you for the citation. [Keith]

8. **For Scheme 1:** [Page 63 of WORD document]
    a. What does "CLEs" after Tier 2 stand for?
    Response: CLEs means Cellular Level Effect corresponding to the Level of Biological Organization in Table 4. The authors have included the abbreviation in Table 4 (first occurrence) and have added a legend to Scheme 1 in the revised manuscript.

    b. Also "OLEs", (presumably occupational exposure limits) is not defined in the text.
    Response: OLEs means Tissue or Organ Level Effect corresponding to the Level of Biological Organization in Table 4. The authors have included the abbreviation in Table 4 (first occurrence) and have added a legend to Scheme 1 in the revised manuscript.

    c. Is the comparator substance the same in tier 2 and tier 3, if so how do the tiers differ?
    Response: [comparator = analog] Determination of the comparator would need to be considered at each Tier. Optimally, it would be best in compiling a weight of evidence that the comparator is the same across the testing tiers; however, there could be technical/testing issues that would make it necessary to use different.

Commented [HT4]: Review for clarity/grammar

d. What is the difference between "evidence does not indicate perturbation of AEC or PU surfactant" and "risk assessment complete"?
Response: If the result of the decision criteria are not met then the evidence does not indicate perturbation of alveolar epithelial cells (AEC) or pulmonary (PU) surfactant. The authors have spelled these out rather than use acronyms in the revised manuscript.
OR
Revise this box in the Scheme to more clearly indicate the decision criteria not met means the chemical does not fit into the Category (for Tier 0 and Tier I) or lung toxicity is not a concern (Tier II and Tier III) ...be more explicit about the hazard conclusion?
[Todd or master of the Scheme]

Risk Assessment Complete indicates that if the if the testing conducted as designated in that Tier provides a point of departure (POD) for the toxic effect that can be used to quantify risk and the risk is acceptable under the regulatory scheme, then the risk assessment can be completed without conducting additional testing in higher tiers.

e. Would the former trigger testing so that you can achieve "risk assessment complete"
Response: Yes; if the if the testing conducted as designated in that Tier provides a point of departure (POD) for the toxic effect that can be used to quantify risk and the risk is acceptable under the regulatory scheme, then the risk assessment can be completed without conducting additional testing in higher tiers.

Commented [TH5]: All comment on Scheme language/clarity by COB Thursday 11/19/20

Some errors:

9. **Page 46:** missing space "Polysorbate 80 (Tween 80)and"
Response: The typographical error has been corrected.

10. **Page 60:** First line, a full stop too much "and. Ulceration"
Response: The typographical error has been corrected.

Message

From: Henry, Tala [Henry.Tala@epa.gov]
Sent: 11/19/2020 1:56:01 AM
To: Sahar_Osman-Sypher@americanchemistry.com; Rick_Becker@americanchemistry.com; Hayes, Michael [hayes.mp@pg.com]; Hillebold, Donna [donna.hillebold@nouryon.com]; ljovanovich@stepan.com; Keene, Athena M. [Athena.Keene@AftonChemical.com]; Kennedy, Wayne [wayne.kennedy@aftonchemical.com]; Moors, Stefan [stefan.moors@basf.com]; Ogden, Julianne [Julianne_Ogden@americanchemistry.com]; Skulsky, Joseph [JSkulsky@stepan.com]; Tveit, Ann [Ann.Tveit@basf.com]; Rose, Jane [rose.jl@pg.com]; Tremblay, Raphael [tremblay.r.2@pg.com]; Stedeford, Todd [Stedeford.Todd@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]; Jarabek, Annie [Jarabek.Annie@epa.gov]; Irwin, William [Irwin.William@epa.gov]; amyjc@piscltd.org.uk; Dr. Monita Sharma [monitas@piscltd.org.uk]
CC: Henry, Tala [Henry.Tala@epa.gov]
Subject: RE: Surfactants Manuscript Path Forward on Peer Reviewer Comments
Attachments: Response to Reviewer Comments_11-18-20_pm.docx; draft manscript general surfactants - 28 August 2020.ver.1_Rev1_11-18-20_pm.docx

Importance: High

Hi all,
I apologize for forgetting to send the versions this morning, but my bad allowed Wayne & Mike to provide responses to comments and some inserts to the manuscript (both attached—with "_pm" extension). I also went throught the Intro & Risk Assessment under TSCA sections and shortened (some) – additional changes will need to be made after the MPPD modeling.

Please use these versions for further edits/etc....if you used yesterday's version, just send it my way and I will incorporate.

Thanks! Tala

Tala R. Henry, Ph.D.
Deputy Director
Office of Pollution Prevention & Toxics

T: 202-564-2959
E: henry.tala@epa.gov

-----Original Appointment-----
From: Osman-Sypher, Sahar <Sahar_Osman-Sypher@americanchemistry.com>
Sent: Friday, November 13, 2020 1:28 PM
To: Osman-Sypher, Sahar; Rick_Becker@americanchemistry.com; Hayes, Michael; Hillebold, Donna; ljovanovich@stepan.com; Keene, Athena M.; Kennedy, Wayne; Moors, Stefan; Ogden, Julianne; Skulsky, Joseph; Tveit, Ann; Rose, Jane; Tremblay, Raphael; Stedeford, Todd; Henry, Tala; Salazar, Keith; Jarabek, Annie; Irwin, William; amyjc@piscltd.org.uk; Dr. Monita Sharma
Subject: Surfactants Manuscript Path Forward on Peer Reviewer Comments
When: Wednesday, November 18, 2020 11:00 AM-12:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: Webex

All:

Please reserve this time to discuss the response plan for the peer reviewer comments received on the surfactants manuscript.

Please use the WebEx information below to join the meeting.

Ex. 6 Personal Privacy (PP) - conference code/call in number

Please Use the WebEx "Call Me" feature using a telephone; or use the "Computer Audio" with a headset.
Call in numbers: Ex. 6 Personal Privacy (PP) - conference code/call in number
Passcode: Ex. 6 Personal Privacy (PP) - conference code/call in number
Global call-in numbers | Toll-free calling restrictions

Thanks, Sahar

Sahar Osman-Sypher | American Chemistry Council
Director, Chemical Products and Technology Division
sahar_osman-sypher@americanchemistry.com
700 2nd Street, NE | Washington, DC | 20002
O: 202-249-6721 C: Ex. 6 Personal Privacy (PP) - personal phone
www.americanchemistry.com

Surfactants Category: The Application of a New Approach Methodology (NAM) for Assessing Inhalation Risks under the ~~Amended~~ Toxic Substances Control Act

Commented [A1]: Its just TSCA now

Tala R. Henry[a,‡], *Keith D. Salazar*[b,‡], *Michael P. Hayes*[c], *Wayne Kennedy*[d], *Athena M. Keene*[d], *Annie M. Jarabek*[e], *Stefan Moors*[f], *Lela Jovanovich*[g], *Jane L. Rose*[c], *Ann Tveit*[f], *Raphaël T. Tremblay*[c], *Richard A. Becker*[h], *Sahar Osman-Sypher*[h], *Patrick D. McMullen*[i], *Scott D. Slattery*[i], *William Irwin*[b], *Marc Odin*[j], *Julie Melia*[j], *Monita Sharma*[k], *Amy J. Clippinger*[k], *and Todd Stedeford*[a,*]

[a] Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC 20460, United States

[b] Risk Assessment Division, Office of Pollution Prevention and Toxics, Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, DC 20460, United States

[c] Procter & Gamble, Company, Inc., St. Bernard, Ohio 45217, United States; Mason, Ohio 45040; Temselaan 100, 1853 Strombeek-Beaver, Belgium

[d] Afton Chemical Corporation, Richmond, Virginia 23219, United States

[e] Health & Environmental Effects Assessment Division, Center for Public Health & Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

[f] BASF Personal Care and Nutrition GmbH, Henkelstrasse 67, 40589 Duesseldorf, Germany; BASF Corporation, Florham Park, New Jersey 07932, United States

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[i] ScitoVation, Durham, North Carolina 27713, United States

[j] SRC, Inc., North Syracuse, New York 13212, United States

[k] PETA International Science Consortium Ltd., London, England

KEYWORDS: Inhalation, Surfactant, New Approach Methodologies, Lung Toxicity, Risk Assessment

ABSTRACT

Commented [A2]: Journal Limit = 300 Words

Abstract as Submitted = 359 Words//2,060Characters

Abstract as Revised = 293 Words//1,709 Characters

Surfactants are chemical substances used in a variety of industrial operations, occupational settings, and consumer products and therefore may result in exposure and toxicity in humans. The Toxic Substances Control Act (TSCA) requires anyone who plans to manufacture (including import) a new chemical substance for a ~~non-exempt commercial purpose~~ to provide the U.S. Environmental Protection Agency (EPA) with a premanufacture notice (PMN) prior to commercialization. ~~Surfactants are a class of chemical substances used in a variety of industrial operations, occupational settings, and in consumer products. Their uses in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to~~

~~humans. While TSCA requires submission of any existing toxicity data, it does not require generation of toxicity data for the purpose of, or prior to, submitting a PMN.~~ TSCA requires that EPA ~~to~~ review the PMN to determine whether the new chemical substance presents an unreasonable risk of injury to human health or the environment. While TSCA requires submission of existing toxicity data, it does not require generation of toxicity data to ~~for~~ submitting a PMN and it mandates that EPA reduce or replace vertebrate animals in testing, to the extent practicable and scientifically justified. EPA therefore relies on ~~several~~ approaches that do not rely on *de novo* toxicity testing to assess chemical risks, including ~~.~~ ~~A~~analogue read-across, in which toxicity data for a chemical of similar structure and activity is ~~are~~ used to assess the new chemical~~,~~ and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) ~~have been used by EPA for decades~~ to assess new chemical substances. This investigation establishes ~~was conducted to identify surfactant chemicals with toxicity data relevant for use in conducting a quantitative human health risk assessment for new surfactant substances and to define~~ a TSCA New Chemical Category for surfactants. ~~Category~~ The category described herein identifies physical-chemical properties to determine chemical inclusion/exclusion in the category, ~~boundaries, which are defined,~~ toxicological analogues suitable for ~~conducting~~ 'read-across' hazard assessment ~~(*i.e.*, hazard identification and dose-response analysis) are identified~~ and a tiered-testing strategy ~~aimed at~~ using new approach methodologies (NAMs) to reduce or replace animal testing ~~is outlined~~. This ~~tiered strategy to defining and evaluating the~~ Surfactant Category provides a pragmatic and scientifically defensible approach ~~to facilitate~~for EPA's review of PMNs for new surfactants and a strategic

testing approach to collect ~~hat provides the~~ data needed to ~~conduct or~~ refine surfactant risk assessments while also meeting the requirements of TSCA to reduce vertebrate testing.

INTRODUCTION

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The Toxic Substances Control Act (TSCA) was amended in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182). The amended TSCA included substantial changes to EPA's authorities and responsibilities, including requirements on EPA to make a determination regarding sufficiency of information, environmental releases and human exposure, and unreasonable risks. The amended TSCA also included provisions mandating EPA to "reduce and replace, to the extent practicable, [and] scientifically justified" the use of vertebrate animals in the testing of chemicals substances. Specifically, TSCA section 4(h) charges EPA with encouraging and facilitating –

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is any compound that reduces surface tension when dissolved in water or water solutions, or which reduces interfacial tension between two liquids, or between a liquid and a solid. [*Hawley's Condensed Chemical Dictionary, R. Lewis, Van Nostrand Reinhold Co.; 1993, pg. 1108.*] ~~a substance that reduces the surface tension of a liquid in which it is dissolved~~. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in industrial processes, occupational settings, and ~~in~~ consumer products (*e.g.*, household cleaning and ~~products,~~ personal care products, *etc.*) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The ~~widespread~~ manufacture, processing and use of surfactants provides opportunities for releases and exposure to humans ~~or environmental receptors~~. The inherent properties of surfactants may induce toxicity if exposures can interfere with biological surfactants or tissues. Certain surfactants are commonly used in a laboratory setting to disrupt cell membranes and denature proteins, which demonstrates the inherent hazards of surfactants. For example, sodium dodecyl sulfate (SDS; Chemical Abstracts Service Registry Number (CASRN) 151-21-3), a strong anionic surfactant, is used at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol (CASRN 9002-93-1), a mild nonionic surfactant, at concentrations up to 1% disrupt cell membranes, while preserving proteins for isolation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

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timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Hazard concerns for surfactants historically focused on their observed environmental effects and potential toxicity to aquatic organisms based on "down the drain" releases and/or presence in effluent from wastewater treatment facilities [ADDIN EN.CITE ADDIN EN.CITE.DATA].The EPA has established chemical categories for nonionic, anionic, and cationic (quaternary ammonium) surfactants based on environmental toxicity concerns [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-

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title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>]. Surfactants may pose a potential hazard to humans, depending on their use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. Mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to "readily penetrate the sandwiched aqueous and lipid barriers of the cornea" [ADDIN EN.CITE <EndNote><Cite><Author>Fox</Author><Year>2008</Year><RecNum>14730</RecNum><DisplayText>[4]</DisplayText><record><rec-number>14730</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017801">14730</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Fox, D.A.</author><author>Boyes, W.K.</author></authors><secondary-authors><author>Klaassen, C.D.</author></secondary-authors></contributors><titles><title>Toxic Responses of the Ocular and Visual System</title><secondary-title>Casarett & Doull's Toxicology - The Basic Science of Poisons, Seventh Edition</secondary-title></titles><pages>665-697</pages><section>17</section><dates><year>2008</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill, Medical Publishing Division</publisher><urls></urls></record></Cite></EndNote>].

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Depending on the conditions of use, the potential for inhalation exposures to workers and/or consumers warrant consideration in quantitative risk assessments. Surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and interfere with the natural pulmonary surfactants and result in reduction in the oxygen content of arterial blood due to impaired gas exchange in the pulmonary region, increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The chemical category boundary for surfactants that may have the potential to present an inhalation hazard has not been previously defined. The toxicity of surfactants by inhalation exposure can vary over several orders of magnitude, based on their chemical properties, although differences in exposure conditions are an important confounder to consider in cross category comparisons. For example, among the available data, a lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) was determined for octylphenoxypolyethoxyethanol, a nonionic surfactant, in a 14-day whole body study[ADDIN EN.CITE ADDIN EN.CITE.DATA] while a LOAEC of 0.08 mg/m^3 in a 4-week nose-only study [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington,

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D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] was observed for didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5), a cationic surfactant and biocide.

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The objectives of the present investigation were to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify inhalation toxicity studies on surfactants that may be used to inform inhalation risk assessments; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing; and (4) establish a tiered-testing strategy that uses NAMs to evaluate new chemistries in the Surfactant Category.

MATERIALS AND METHODS

Systematic Literature Review

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Two literature searches were performed, an initial search from 1950 through November 2016 and a supplemental search up to April 2018. The details of these searches, including the search strategies, search terms, search results and Population, Exposure, Comparison, and Outcome (PECO) criteria used for reviewing the relevance of the identified studies to this evaluation are provided in the Supporting Information file at "Section 1 Systematic Literature Review". These searches were conducted with the primary objective of identifying studies that evaluated the toxicity of surfactants in the respiratory tract of humans or laboratory animals, and at the cellular

level in *in vitro* and *ex vivo* studies. In addition, these searches were used to identify potential NAMs that could inform a tiered-testing strategy for general surfactants that reduces or replaces the use of vertebrate animals in regulatory testing.

Risk Assessment Approaches under TSCA

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Risk Assessment Paradigm

~~The methods for assessing~~Assessment of risks of new chemical substances under TSCA ~~have been developed using science-based approaches, scientific peer review, and refinement of the approaches. EPA conducts risk assessments~~ follows ~~ing~~ the four-step process articulated by the U.S. National Research Council (NRC) in 1983 [11] and reaffirmed several times since its initial release [12, 13]. This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the adverse health or environmental effects, or hazards, that can be caused by exposure to a chemical substance. The dose-response analysis assesses the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects. The exposure assessment characterizes human or environmental exposures, including the magnitude, frequency, and duration, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure components to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these risk assessment components, including the level of detail and complexity of quantitative aspects, may vary across different risk assessments and typically align

with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14738</RecNum><DisplayText>[11]</DisplayText><record><rec-number>14738</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019129">14738</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>40 CFR Part 720 - Premanufacture Notification</title><secondary-title>Code of Federal Regulations</secondary-title></titles><periodical><full-title>Code of Federal Regulations</full-title></periodical><pages>https://www.law.cornell.edu/cfr/text/40/part-720</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], companies are required to submit a PMN along with available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances.

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives hazard data for new chemical substances. An analysis of toxicity data submitted to EPA from 2004 through 2012 for new chemical substances found that only about 15% of the PMN submissions included health hazard data; the majority of which was that information was for acute toxicity ~~(e.g., 24-hour dermal toxicity study with a 14-day post-administration observation period)~~ and irritation ~~(e.g., 4-hour dermal irritation/corrosion with a 14-day post-administration observation period or 24-hour eye irritation/corrosion with a 21-day post-administration observation period)~~ in laboratory animals. TSCA provides EPA with the authority to require the generation ~~and submission~~ of additional data when the information included with the PMN ~~coupled with that available to EPA risk assessors from predictive modeling, read-across, internal archives, *etc.*~~ is insufficient to permit a reasoned evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must first take into consideration reasonably available existing information, including toxicity information (*e.g.*, in the scientific literature or internal archives, *etc.*; computational toxicology and bioinformatics (*e.g.*, predictive modeling, read-across); and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

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Given the historical lack of hazard data for new chemical substances, EPA has, for decades, employed a number of approaches that do not rely on *de novo* toxicity testing. These approaches

include computational toxicology (*e.g.*, predictive models and expert systems), analogue[1] read-across wherein available toxicity data for a chemical of similar structure and activity are used to assess the new chemical substance lacking data, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. EPA has a ~~'s current~~ chemical categories document on surfactants entitled "TSCA New Chemicals Program (NCP) Chemical Categories" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] that includes information for

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[1] In the context of this article, an analogue is a chemical substance identified based on its physicochemical and toxicological properties, as one that has undergone evaluation, as stated above, and determined to be an acceptable toxicological analogue for read across to the new chemical substance. An analogue may be directly used in read-across for informing a quantitative risk assessment on a new chemical substance.

anionic, nonionic, and cationic surfactants; however, ~~these were previously~~the categories are ~~developed and~~ defined only for ~~an~~ environmental toxicity considerations.

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The integration of these methods with NAMs to advance testing strategies has been recognized by Dellarco *et al.* [ADDIN EN.CITE ADDIN EN.CITE.DATA] and is consistent with the vision articulated in the 2007 report by the NRC in "Toxicity Testing in the 21st Century: A Vision and Strategy" [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[16]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>]. EPA defines NAMs "as a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14844</RecNum><DisplayText>[17]</DisplayText><record><rec-number>14844</rec-number><foreign-

keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1597332016">14844</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA Program</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>39, https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf</pages><volume>EPA-740-R1-8004</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]

Dose-Response Analysis

~~In the absence of test data on new chemical substances, EPA relies on read-across methods using an analogue or a category of analogues in the absence of test data on the new chemical substance to identify hazards and conduct d~~Dose-response analysis is conducted, ~~whether on a new chemical substance or an appropriate analogue,~~ to identify a point of departure (POD), *i.e.*, a dose or concentration that marks the beginning of a low-dose extrapolation. In the absence of test data on new chemical substances ~~T~~toxicity data for analogues are used to identify a POD, such as a no observed adverse effect (concentration) level (NOAE(C)L) or lowest observed adverse effect (concentration) level (LOAE(C)L, for assessing risks of the new chemical substance. This

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POD can also be the lower bound on dose (or concentration) for an estimated incidence or a change in response level calculated by a dose-response model such as those available in EPA's benchmark dose software (BMDS), *e.g.*, the BMCL for an observed incidence or change in level of response [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>14744</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14744</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019975">14744</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Benchmark Dose Technical Guidance</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>99, https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf</pages><volume>EPA/100/R-12/001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>]. ~~EPA's current chemical categories document on surfactants entitled "TSCA New Chemicals Program (NCP) Chemical Categories"~~[ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] ~~includes information for anionic, nonionic, and cationic surfactants; however, these were previously developed and defined only on environmental toxicity considerations.~~

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EPA's ~~has also developed~~ guidance to improve the science underlying the animal-to-human uncertainty factor (UF_H), which ~~and~~ provides generalized procedures for deriving dosimetric adjustment factors (DAFs) to perform interspecies extrapolation [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 20]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

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20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>] is also used in dose-response analysis. Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the human equivalent concentration (HEC). Application of a DAF in the calculation of an HEC is considered to address the toxicokinetic (TK; *i.e.*, absorption, distribution, metabolism, and excretion) aspects, but not the toxicodynamic (TD; *i.e.*, mode of

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action) component, of the ~~animal-to-human uncertainty factor~~ (UF_H) (*i.e.*, to estimate from animal exposure information the human exposure scenario that would result in the same dose as achieved in the animal to a given target tissue) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>]. This operational derivation of a DAF involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle, reactive gas, or volatile organic compound) coupled with consideration of the location and type of toxic response. These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (*e.g.*, to a weekly average based on number of h/d and d/w).

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For interspecies extrapolation of particle exposures, the Regional Deposited Dose Ratio (RDDR) model developed by EPA can be used to derive a DAF. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. EPA's RDDR model allows calculation of RDDR estimates in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic [ET], tracheobronchial [TB], pulmonary [PU], thoracic [TH], total respiratory tract [RT] and extra-respiratory [ER] regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (*i.e.*, the Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or

GSD, and density), and species-specific parameters for both animals and humans including ventilation rates and regional surface areas of the respiratory tract. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The DAF is then applied to the duration-adjusted POD to arrive at the HEC of the POD (POD_{HEC}). The EPA's RDDR model was used herein to calculate HEC values from the aerosol exposures to laboratory animals available for each of the surfactant classes.

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After an analogue(s) is identified, the strengths, limitations, and uncertainties associated with the use of the ~~substance~~analogue(s) to predict the hazards and POD for the new chemical substance under evaluation are considered when deriving a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant UFs to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL to a NOAEL [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[19, 21]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A

Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. EPA prefers using existing information to develop data-derived extrapolation factors (DDEFs) or chemical specific adjustment factors (CSAFs) rather than relying on default values [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><DisplayText>[21]</DisplayText><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. This investigation includes several approaches to derive DDEFs for use in assessing new surfactant chemical substances.

Exposure Assessment

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In assessing new chemical substances, generally new chemical substances do not have occupational exposure monitoring data or consumer exposure data; therefore, EPA typically evaluates occupational exposures first, given that these represent the highest exposure estimates. Therefore, this evaluation focused on occupational exposures, recognizing that consumer exposures would also be considered, if applicable. EPA develops exposure estimates for workers using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER)

model. ChemSTEER estimates exposure as daily acute potential dose rates (PDRs) or lifetime average daily doses (LADDs). The PDR represents average exposure over an 8-hour workday, whereas the LADD estimates long-term exposures to the chemical substance and is averaged over a lifetime exposure of 75 years. The PDR, an initial conservative exposure estimate, is considered to be the more appropriate dose-metric for estimating risks to surfactants because surfactants are surface-active at the point of exposure and effects in the respiratory tract occur rapidly following exposure. This assumes that neither the chemical nor its damage accumulate or distribute to systemic compartments. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR values are 1.875 mg/kg-bw/day for inhalable aerosols or 0.625 mg/kg-bw/day for respirable aerosols as shown in [REF _Ref46930162 \h * MERGEFORMAT] [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2015</Year><RecNum>14745</RecNum><DisplayText>[22]</DisplayText><record><rec-number>14745</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021217">14745</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>ChemSTEER User Guide, Chemical Screening Tool for Exposures and Environmental Releases</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>403, https://www.epa.gov/sites/production/files/2015-

05/documents/user_guide.pdf</pages><dates><year>2015</year></dates><urls></urls></recor

d></Cite></EndNote>].

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Table [SEQ Table * ARABIC]. Default values used for calculating the daily acute potential dose rate (PDR).

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Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg-bw	kg-bw

[a] Cm may also be adjusted for the mass concentration of the chemical with a permissible exposure limit (PEL) in air (based on the U.S. Occupational Safety and Health Administration [OSHA] PEL – time-weighted average [TWA]; where: KCk = the mass concentration limit of total particulate in air (mg/m^3) with a default of 15 mg/m^3 for inhalable and 5 mg/m^3 for respirable, Ys= the weight fraction of chemical in particulate ($0 < Ys \leq 1$), Ypel=the weight fraction of chemical or metal in particulate with a known PEL ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

The PDR is calculated using an exposure regimen for a default worker of 8 hours/day and 5 days/week, unless chemical-specific manufacture, processing or use information are provided in the PMN. The exposure conditions in laboratory animal studies often do not reflect occupational exposure scenarios; therefore, a duration adjustment and a DAF (*i.e.*, RDDR value) are applied to the POD to derive HECs for exposed human populations according to Agency methods [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Therefore, the interspecies extrapolation is performed using particle deposition models that adjust for the aerodynamics of the given particles in the different airway architecture between the

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species and using species-specific physiologic parameters such as ventilation. The occupational exposure is characterized with human ventilation rates during exertion (work) and exposure durations appropriate to the specific occupational setting and chemical use scenario.

Risk Characterization

~~Risk characterization is the final, integrative step of risk assessment.~~ EPA's Risk Characterization Policy defines risk characterization as the integration of information from the hazard and exposure components of the risk assessment into an overall conclusion about the existence (or lack of) ~~risk that is complete, informative, and useful for decision-making. The risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of)~~ human health or ecological risks [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2000</Year><RecNum>14747</RecNum><DisplayText>[23]</DisplayText><record><rec-number>14747</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021806">14747</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Risk Characterization</title><secondary-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>189, https://nepis.epa.gov/Exe/ZyPDF.cgi/40000006.PDF?Dockey=40000006.PDF</pages><volume

>EPA 100-B-00-002</volume><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>]. It is recognized that ~~As described in EPA's Risk Characterization Handbook~~ "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized and the level of information contained in each risk characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios. The MOE is the HEC derived from a POD for a health endpoint (from hazard assessment) divided by the exposure concentration for the scenario of concern (from exposure assessment). The calculated MOE is compared with a benchmark MOE to evaluate whether there is an adequate margin between human exposure estimates and the HEC. When the MOE is less than the benchmark MOE, there is a possibility of human health risks. On the other hand, risks are not expected ~~negligible concerns would be expected~~ if the MOE exceeds the benchmark MOE. ~~The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios.~~

In summary, in developing a risk assessment for new chemical substances under TSCA section 5, EPA uses empirical data or analogues, to identify a POD(s) and to develop an exposure estimate for use in the evaluation. The hazard assessment in combination with the exposure assessment is used to calculate an MOE, which is compared to the benchmark MOE to identify potential risks. The risk characterization is used to inform the TSCA "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

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An initial search of PubMed identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles that met the PECO criteria were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc*., and were included for full text review. Of the 60 articles evaluated through full text screening, 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search of PubMed and Embase, 1247 articles (combined) were identified. Following title and abstract screening, 1217 of these articles were excluded because they did not meet the PECO criteria, whereas 25 met the PECO criteria and were selected for full text review. An additional 10 studies that met the PECO criteria were found by additional hand searching) and were selected for full text screening, which resulted in 35 articles that were

identified for review; ten articles were deemed irrelevant and excluded. A total of 25 articles were identified from both searches, one was excluded because it was in a foreign language and the remaining 24 articles are summarized in Table 8 in the Supporting Information file at "Section 1 Systematic Literature Review".

The information identified in the systematic review was used to determine Category Boundaries and subcategories, to summarize the health effects of surfactants under the section on Hazard Identification, and to identify potential NAMs for use in the Tiered-Testing Strategies.

Category Boundaries

The following structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) [ADDIN EN.CITE ADDIN EN.CITE.DATA]:

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance is capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test concentration of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less (as measured using a standard method).

Commented [A22]: Reviewer 2: How is this measured? WAYNE/MIKE?
ADD A BRIEF DESCRIPTION/ PARENTHETICAL AND REFERENC HERE

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The Surfactants Category is further defined into three general subcategories including nonionic, anionic, and cationic substances. Amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, anionic and cationic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging from 6.6 to 7.1 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The pKa for each component of an amphoteric surfactant should be evaluated within this pH range and the assessment should be conducted on the predominant components. The non-ionized fraction for acids/bases is calculated as follows:

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pH-pKa}})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pKa-pH}})$$

Where the pH represents the physiological pH in the lung lining fluid (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine).

Nonionic surfactants are identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. Examples of ~~For example, octylphenoxypolyethoxyethanol, a~~ common

nonionic surfactants and the range of corresponding surface tension measurements associated with them ~~octylphenol EO surfactant, and Polysorbate 80 (or Tween 80; CASRN: 9005-65-6), another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units,~~ are shown in [REF _Ref47613375 \h * MERGEFORMAT]. ~~The surface tensions of octylphenoxypolyethoxyethanol and Polysorbate 80 range from 30-31 mN/m to 37-96 mN/m, respectively ([~~ REF _Ref47613375 \h * MERGEFORMAT]~~)~~[ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware, A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].

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Anionic surfactants are identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates,

alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). An example anionic surfactant, SDS, has a reported surface tension of 35 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]).

Cationic surfactants are identified as any chemical substance with a net positive charge that meets the Surfactant Criteria ~~(e.g., alkylammonium chlorides and benzalkonium chlorides). Benzalkonium chloride (BAC; CASRN 8001-54-5) and didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5) are R~~representative members of this subcategory, with surface tensions ~~of 37 mN/m and 25.82 mN/m ([~~ REF _Ref47613375 \h * MERGEFORMAT ~~]), respectively~~ are provided in Table 2. It is noted that BAC and DDAC also possess biocidal properties.

Typical commercial surfactants (nonionic, anionic, and cationic) are non-volatile[3] liquids or solids. This category framework focuses on exposure *via* aerosol forms (*i.e.*, both airborne droplets and solid particles, including the hygroscopic variety) of these surfactants. While the commercial use of volatile surfactants is unlikely, it should be noted that this framework is not applicable to any substances that qualify as surfactants and are volatile under the conditions of use.

[3] Volatility is considered as part of the ChemSTEER modeling, wherein a vapor pressure of 1.3×10^{-04} kPa is the cutoff for gases/vapors.

Table [SEQ Table * ARABIC]. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cationic Subcategorization.

Nonionic Surfactants					
Chemical Name in Text	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**
		Hydrophobic group(s)	**Hydrophilic group(s)**	**Surface Tension**	**Critical Micelle Concentration (CMC)**
formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol Defomaire Alevaire Tyloxapol CASRN: 25301-02-4	CAS Name: formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol	multiple octyl phenol groups	multiple polyoxyethylene (9) units	~37 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,	0.038 g/L or 0.0038 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sch

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				H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-	ott, H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface